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The Influence of the pH-value on the Growth of *Brevibacterium epidermidis* in Continuous Culture

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***Brevibacterium epidermidis* is a major component of the bacterial flora of certain skin surface biotopes, characterized by a comparatively high pH-value. The presence of *Brevibacterium epidermidis* seems to be linked to the production of malodour. Skin surface pH has been found to be a major factor of bacterial growth on the skin.**

In order to find out if this might also apply to *Brevibacterium epidermidis*, this microorganism was grown in vitro in continuous culture using a chemostat.

Specific growth rate and density of colony forming units were well correlated. While the organism grew readily from pH 5.5 to 8.5, this was not the case with a pH of 5.0.

Thus pH-shifts induced by cosmetic procedures can only prevent unpleasant body odour due to abundant growth of bacteria if the pH-value is decreased to 5.0 or less. *Key words: skin surface pH; body odour.*

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Aerobic coryneform organisms are gram-positive pleomorphic rods. These bacteria constitute major components of the regular bacterial flora of human skin (1). In the past, the coryneforms have been classified in different ways (2–6). Cell wall analysis showed that only 60% of human cutaneous coryneform bacteria belong to the *Corynebacterium* genus (7). Twenty per cent of the coryneform organisms were classified as *Brevibacterium* species and 20% belonged to other groups. Thus, the importance of *Brevibacteria* as a major component of the human skin surface flora is obvious.

Brevibacterium species do not only produce peptides with antibiotic properties (8), which are possibly responsible for the prevalent type of flora in the sense of a coryneform- or coccidominated flora, but are also said to play an important role in the production of body odour, possibly related to methanethiol production (9). Interactions between dermatophyte fungi and *Brevibacteria* due to the selection of penicillin-resistant *Brevibacterium epidermidis* have also been described (10).

According to previous studies, the surface of unoccluded human skin is acidic (11, 12) recent experimental findings amounting to pH 4.4 to 5.8 (13). This, however, does not apply to the human axilla. Here even pH-values higher than 7.0 are measured (12). As *Brevibacteria* predominantly grow in this area and as the human axilla is one of the most important areas for the production of skin odour, it is important to evaluate the growth of *Brevibacteria* at various pH-values. In fact, the skin surface pH is open to changes according to the pH-value of skin cleansing agents used, as is the bacterial flora accordingly (13).

Continuous culture experiments using a chemostat seem suitable for analysing the relationship between pH-value and bacterial growth (14, 15). In the following, continuous culture experiments with *Brevibacterium epidermidis* are described.

MATERIALS AND METHODS

Microorganism

Brevibacterium epidermidis DSM 20569 (Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany) was chosen for investigations.

Culture media

Trypticase soy broth (BBL, Heidelberg, Germany) was used, essentially containing pancreatic digest of casein 17 g, papaic digest of soybean meal 3 g, sodium chloride 5 g, dipotassium phosphate 2.5 g and dextrose 2.5 g per liter.

Culture conditions

All experiments were performed in a Biostat M chemostat (Braun-Melsungen AG, Melsungen, Germany). A pump FE 411 and a multichannel-plotter Jumo comp PD (Braun-Melsungen AG) were also used.

The following parameters were pre-fixed: gas supply, flow of broth through the vessel, rotation speed. Temperature, volume of broth in the culture vessel, pH-value and foam production were kept constant.

The following parameters were chosen: air flow: 0.2 l/min, temperature: 37°C, rotation speed: 800 rpm, flow of broth through the vessel: 18 ml/h, volume of the culture vessel: 900 ml.

Silicon-Antischaum-Emulsion M-30 reinst (Serva, Heidelberg, Germany), diluted with destilled water 1:10, was used as anti-foam substance.

Determination of bacterial density

Bacterial density, expressed as the number of colony-forming-units (CFU) per ml was determined by repeated dilution of defined volumes and consecutive inoculation of trypticase soy yeast agar. This medium contains per liter: trypticase soy broth (BBL) 30 g, yeast extract (Difco Laboratories, Detroit, Michigan, USA) 10 g, Bacto-Agar (E. Merck, Darmstadt, Germany) 20 g, Tween 80[R] (E. Merck) 5 ml. Samples were taken at the beginning of each experiment. Later on, samples were taken generally at 5, 7, 9, 11, 13, 25, 29, and 37 h. Later points of time, if addressed at all, are depicted in the graphs.

pH-values

Each value was kept constant by the addition of 1 M NaOH or 1 M HCl. Experiments at a pH-value of 4.5, 5.0, 5.5, 6.5 were performed in duplicate, reflecting the usual skin surface pH-range. Experiments at pH-values of 7.5, 8.5 and 9.5 were performed once in addition.

Determination of growth behaviour

Specific growth rate was determined by drawing a compensation line between the 5th and the 25th hour. Specific growth rate was obtained as the reciprocal value of the time required for doubling bacterial counts.

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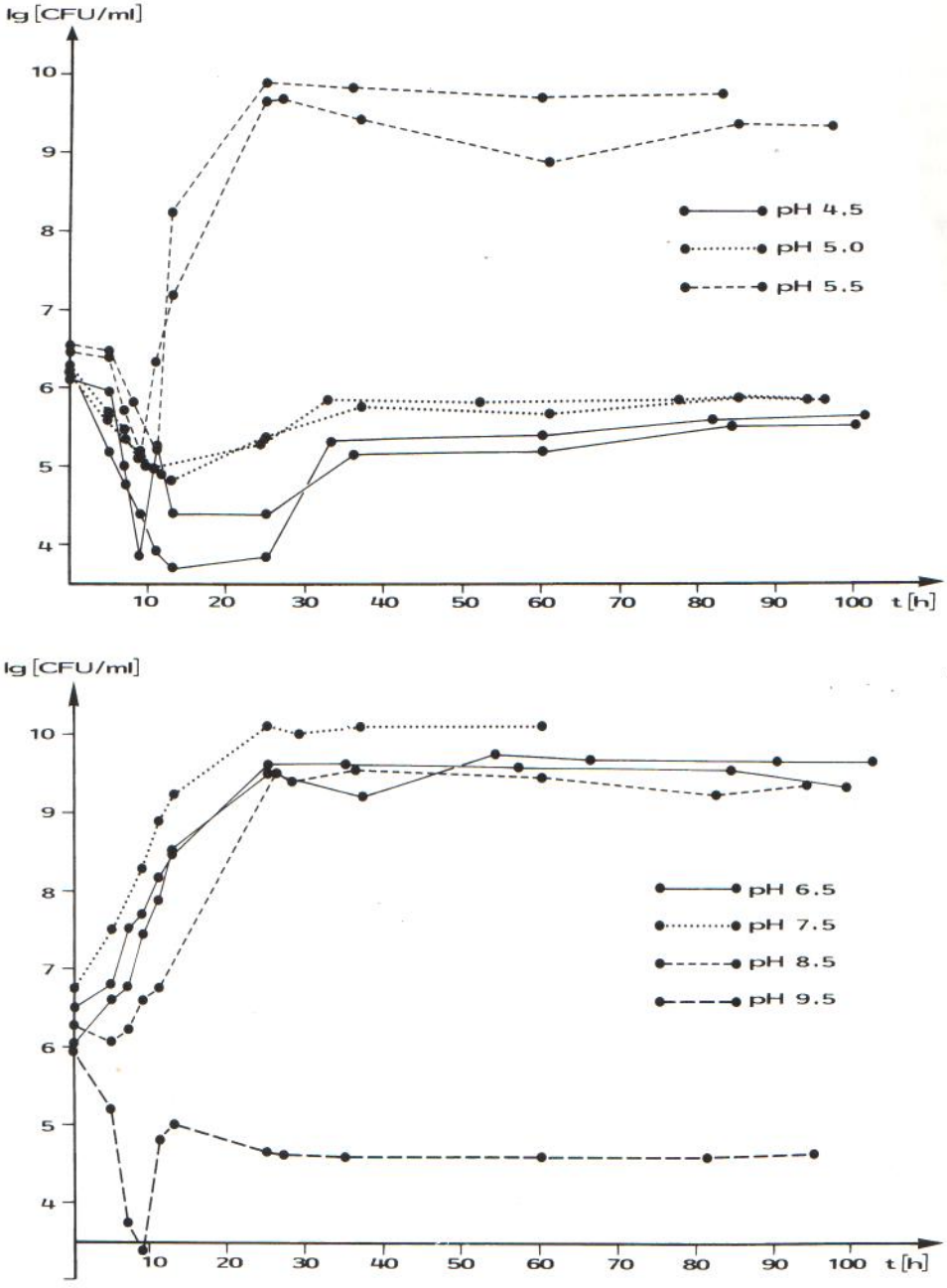

Fig. 1a,b. Density of *Brevibacterium epidermidis* DSM 20659 in continuous culture at different pH-values.

The average of the bacterial densities between the 3rd and 5th day was taken as the plateau in the steady state.

RESULTS

The changes of bacterial density over time at the various pH-values are depicted in Fig. 1a,b. The specific growth rates differed a lot according to the pH-value given: a specific growth rate exceeding 0.4 (h^{-1}) was found within the pH-range from 5.5 to 8.5. At a pH of 9.5 the specific growth rate was negative. At pH 5.0 the specific growth rate amounted to about 0.1; at 4.5 it was about 0.

Unpleasant odour production was recognized most markedly at a pH-value of 7.5, where the highest bacterial density could be observed.

DISCUSSION

When one looks at the figures, the broad spectrum of good growth conditions is obvious. The specific growth rate is markedly higher between pH-values of 5.5 to 8.5, and so is the count of colony-forming units during the plateau phase. This is in clear contrast to the results observed in chemostat experiments with *Staphylococcus epidermidis*, another typical microorganism of the resident flora of the human skin surface (16).

During the first 15 h, a reduction of colony-forming-units can been seen at pH-values of 4.5, 5.0 and 9.5 (Fig. 1). A possible explanation could be the lower specific growth rate at these pH-values. Nevertheless, at these pH-values a plateau and thus a steady state can be reached later on, although at a lower level.

Optimum growth of *Brevibacterium epidermidis* at pH 5.5 to

8.5 reflects the higher frequency as well as density of coryneform bacteria in occluded skin areas (17), such as the axilla, also demonstrating such a pH-spectrum. Correspondingly, markedly lower growth rates at 5.0 and 4.5 in vitro reflect the scarcity of the organism on more acidic unoccluded skin, e.g. of the forearm.

Coryneform bacteria as *Brevibacteria* are not only obtained from human skin, but also from dairy sources. Some of the microorganisms from either source produce methanethiol (9). This contributes to the familiar flavour of some types of cheese, as well as unpleasant body odour (9). However, other causes of malodour, such as testosterone metabolites, must also be considered (18, 19).

The present experiments demonstrate a limited possible influence of changing the skin surface pH by cosmetic intervention. In fact, slight changes in skin surface pH towards the alkaline have been brought about by the repeated application of alkaline soap, which was followed by an increase in the density of *Propionibacteria* (13). With *Propionibacteria*, however, there is a critical difference in the specific growth rate between 5.5 and 6.0 (16). Thus, a minute shift of the pH, which is in fact readily feasible, may lead to a major change in the bacterial skin flora (13).

The idea of the induction of a change of the skin surface pH has also already been discussed, addressing the optimum composition of a deodorant (20). Unfortunately, a corresponding product containing triethylcitrate proved inefficacious except for its additional perfume component (21). Although this was probably due to the lack of any pH-change brought about by the given preparation, one has to ask if such a big shift as from about 7.0 to 5.0 can be expected from any cosmetic.

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