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Growth inhibition of coryneform bacteria by a mixture of three natural products—Farnesol, glyceryl monolaurate, and phenoxyethanol: HGQ

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Synopsis

Axillary malodor is produced by secretions of the apocrine sweat glands that are contaminated by coryneform bacteria. One of the mechanisms of deodorant action is to inhibit bacterial growth. This investigation was designed to study the bactericidal effect of the mixture of three natural products, HGQ,* on wild strains of axillary coryneform bacteria. Thirty corynebacteria species could be isolated and identified biochemically from 530 underarm swabs. Their sensitivity to the HGQ mixture was determined by means of the minimum bactericidal concentration. It ranged from 0.025% to 1% HGQ. 47% of the strains were inhibited by less than 0.1% HGQ, 30% by less than 0.3%, and 23% by less than 1.0% HGQ. The sensitivity to HGQ did not correlate with specific corynebacteria strains but with the survival time of the strains, i.e., the most sensitive strains had the shortest survival times at 4°C, both on blood agar and in the stab culture.

Our studies show that HGQ, a mixture composed of three products occurring in plant or animal species, farnesol, glyceryl monolaurate, and phenoxyethanol, kills corynebacteria and that it can be recommended for use as an effective deodorant, as has already been confirmed by its successful use in practice.

HGQ and its components are biologically degradable to more than 60% within 28 days. Only CO_2 and H_2O are formed, since there is no nitrogen contained in HGQ.

Furthermore, this finding indicates that although the synergistically acting HGQ mixture has a selective bactericidal effect, it is degraded naturally after use, which is not true of the first generation of deodorants.

INTRODUCTION

The underarms play an important role in the generation of body odor. Odor is generated here from secretions of the apocrine sweat glands, which are primarily contaminated by coryneform bacteria (20). C_3-fatty acids, (iso)butyric acid, isovaleric acid, and androgen steroids such as 16,5-Ó-androsten-3β-ol and 16,5-Ó-androsten-3-1 have been identified as the odorous substances. The latter are excreted in odorless axillary sweat as water-

* Farnesol Plus®, Dragoco Gerberding & Co. GmbH, D-3450 Holzminden, Germany.

soluble sulfates or glucuronides and then liberated by hydrolytic enzymes of bacteria and/or the skin as volatile steroids.

A gas chromatographic analysis of axillary sweat, performed parallel to a sniff test with perfumers, gave 20 different odorous substances. A few by these substances characterized only by retention times occurred in all persons studied (2). Moreover, amino acids with a characteristic odor have been identified in eccrine sweat (15).

Sweat secretion, the bacteria population, and a moist environment are the three major components contributing to odor production by the skin (4,6,14,21,22). The bacteria flora of the skin vary within broad limits both qualitatively and quantitatively (5,14). In a review, the relationships between the bacteria population, the host, and the environment have already been described (7).

From the foregoing it becomes understandable why it is possible to inhibit sweat odor by different mechanisms. These include:

- inhibition of sweat secretion by systemic administration of sedatives, ataractics, parasympatholytics, and saluretics
- application of topical antiperspirants, such as formaldehyde, glutaraldehyde (danger of sensitization), and formulations containing aluminum hydroxycholoride and tannins
- binding of odorous substances by mixtures of zinc ricinoleate and other zinc compounds that act synergistically (17)
- environmental control, e.g., by body hygiene (soap or surfactants), and by absorbent, loose underwear
- deodorizing by means of an antibacterial therapy with strong disinfectants such as halogenated phenol compounds or quarternary ammonium compounds that influence virtually all of skin flora in the same way (12)

Inhibition of esterases is an alternative mechanism of deodorant action. Glyceryl triacetate, triethyl citrate, and other rapidly saponifying esters represent substances acting according to this mechanism. However, most other esters are bacteriologically inert, i.e., have no measurable antibacterial effect, when tested according to conventional methods.

The aryl sulfatases and β-glucuronidases can also be inhibited by Cu^{++}- and Zn^{++}-compounds. For example, even concentrations of 10–100 μ Cu or zinc glycinate have an effect (3). If proliferation of bacteria is prevented by antimicrobial substances, the production of skin odor caused by bacterial decomposition of sweat is also largely reduced. However, it is still possible that the deodorant effect could be a result of a regulatory role of the substance in the biochemical processes on the skin surface.

For years the deodorant HGQ, which is recommended as a natural synergistic complex of active substances (9), has proved successful in everyday use. It is offered as a concentrate as well as a 50% solution in dipropylene glycol or ethanol, and consists of three individual components:

- 34% *farnesol,* which has been identified in cotton bud oil, cabreuva oil, musk seed oil, neroli oil, tuberose blossom oil and other volatile oils
- 11% *glyceryl monolaurate,* found in the feather fat or marabous (Leptoptilos crumeniferus)
- 53% *phenoxyethanol,* which occurs in tropical fruits, in Cichorium endivia and in Camellia sinensis (green tea)

In a concentration of 0.3%, HGQ completely inhibits the growth of *Staphylococcus aureus, Staphylococcus epidermidis,* and *Propionibacterium acnes* (10). The present study was designed to test the bactericidal properties of HGQ against wild strains of corynebacteria isolated from human axillary swabs. Based on biochemical characterization, 30 species of coryneform bacteria were identified from 530 human axillary swabs. These were tested for their sensitivity to HGQ.

MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF CORYNEBACTERIA STRAINS

Smears freshly taken with cotton swabs from axillary skin were plated on blood and endoagar. After incubation for 24 and 48 hours at 37°C, potential corynebacteria strains were isolated as individual colonies. They were identified by their typical macromorphological appearance, being usually gray, opaque colonies, and by gram staining as gram-positive rods. Different strains were identified by examination of the following properties: catalase and oxidase production capacity, β-hemolysis on sheep blood agar, nitrate reduction, pigment formation, ureases, gelatin hydrolysis, mobility, esculin hydrolysis, serum response, and glucose, lactose, maltose, rhamnose, arabinose, trehalose, saccharose, xylose, and manitol fermentation. Tests were performed and interpreted according to methods described by Coylectal and Lipsky (1), Lenette *et al.* (13), and Lipstick *et al.* (16). Reference strains in the tests were a *C. diphtheriae* Park William-8-strain and a *C. pseudotuberculosis strain.* The purity of the strains to be identified was checked on blood agar plates before and after application of the inoculum to the test series.*

TESTING THE SENSITIVITY OF IDENTIFIED SPECIES OF CORYNEBACTERIA STRAINS TO HGQ

The only feasible method proved to be determination of the minimum bactericidal concentration (MBC). 10, 3, 1, 0.5 and 0.25% HGQ stock solutions were prepared in 70% ethanol. Nine milliliters of glucose nutrient broth (Berlin-Weißensee Institute of Immunopreparations and Nutrient Media) were added to 1 ml of each of the HGQ stock solutions, giving a final HGQ concentration of 1 to 0.025% and an ethanol content of 7%. An inoculating loop was used to inoculate the 6-h preculture, which had been adjusted to 1 million viable microorganisms/ml. An inoculated glucose nutrient broth tube with 7% ethanol served as the positive control, and uninoculated tubes of each of the HGQ concentrations were the negative controls. To ensure a maximum homogeneous distribution of HGQ in the nutrient medium, the tubes were shaken at a moderate rate during the entire 18-h incubation period at 37°C. Aliquots from the shaken culture tubes were spread on blood agar (5% human blood) by means of an inoculating loop. After incubating for 48 h at 37°C, those smears in which less than 0.1% of the original inoculum could be counted as individual colonies were taken as the MBC.

* We are grateful to Dr. Med. Lehmann of the Institute of Medical Microbiology and Epidemiology of the University of Leipzig for his assistance with species identification.

RESULTS

The study of 530 axillary swabs and their bacteria isolates reveals a total of 30 coryneform bacteria (Figure 1). These include *C. jeikeium,* pathogenic bacteria resistant to antibiotics. All of the C. species listed in Table I are virtually destroyed in the suspension test in the range of 0.025% to 1% HGQ (99.99% reduction of the initial microbial count).

Table I shows that six of the strains studied were already inactivated by a HGQ concentration below 0.025%, another 8 below 0.1%, 9 below 0.3%, and 7 strains below a HGQ concentration of 1%. Although no correlation could be determined between the HGQ sensitivity and specific corynebacteria, there was definitely one between the HGQ sensitivity and the survival time of individual strains. Strains 1–6, which were especially sensitive to HGQ, had the shortest survival times, both on blood agar plates and in the stab culture when stored in the refrigerator.

Assessment of the inhibitory (deodorizing) effect of a formulation on skin odor should not be based on the antimicrobial range of the preparation alone. It should also include other specifically use-related tests to enable decisions appropriate to the composition of the preparation.

Deodorants are usually assessed by means of the sniff test (8), a sensory method that is based on the evaluation of the underarm odor of a consumer group consisting of at least 30 subjects by a panel of experts with a reliable judgment of odors. The active complex, HGQ, whose effect is to be evaluated in this case, is composed of three natural products that possess some bacteriostatic properties. When the whole group is evaluated after regular use of deodorants and the formulations containing the active ingredient are compared with those containing no active ingredient, the following results are obtained:

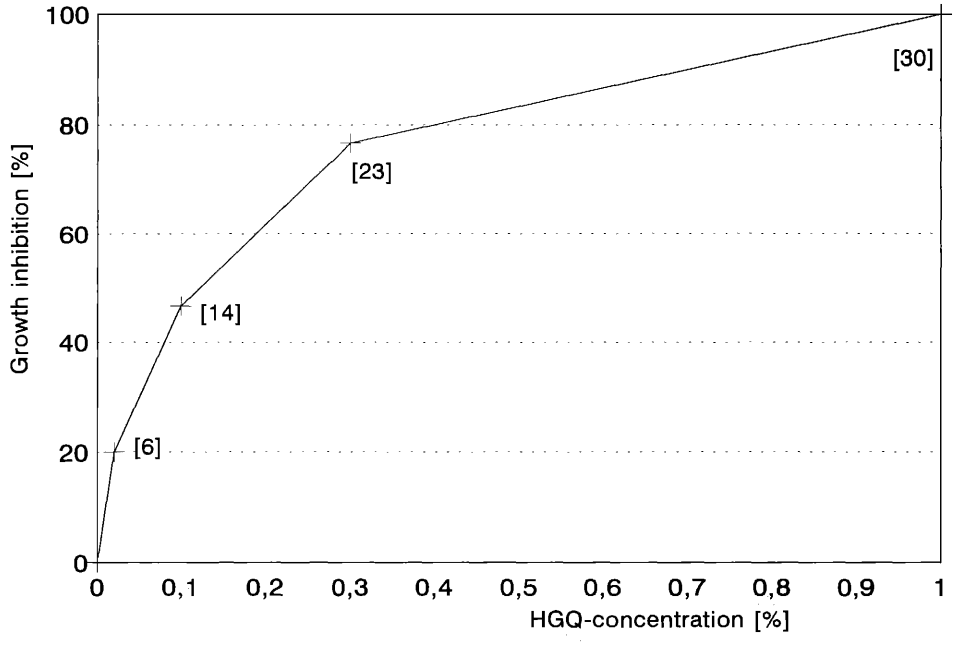

Figure 1. Minimal bactericidal concentration of HGQ against 30 strains of corynebacteria.

Table I
Minimum Bactericidal Concentration (MBC) of HGQ for 30 Species of Coryneform Bacteria Isolated From Human Underarms

Strain no.	Species	MBC (% HGQ)
1	*C. jeikeium*	<0.025
2	*C. pilosum*	<0.025
3	*C. pilosum*	<0.025
4	*C. pyogenes*	<0.025
5	Group I	<0.025
6	B-1	<0.025
7	*C. jeikeium*	0.1
8	*C. kutscheri*	0.1
9	*C. pilosum*	0.1
10	Group I	0.1
11	Group I	0.1
12	Group I	0.1
13	Group I	0.1
14	B-3	0.1
15	*C. cystidis*	0.3
16	*C. jeikeium*	0.3
17	*C. minutissimum*	0.3
18	*C. xerosis*	0.3
19	*C. xerosis*	0.3
20	*C. xerosis*	0.3
21	Group A-4	0.3
22	Group F-2	0.3
23	Group I	0.3
24	*C. jeikeium*	1.0
25	*C. xerosis*	1.0
26	Group I	1.0
27	Group I	1.0
28	Group I	1.0
29	Group I	1.0
30	B-1	1.0

the group with strong body odor, i.e., those really in need of a deodorant, give better results with soap containing the active ingredient than with a placebo (Table II, Figure 2). The differences are highly significant. The deodorant effect is comparable to that obtained with soaps containing trichlorocarbanilide (24).

It can thus be concluded (Table III) that the deodorant effect of HGQ is also comparable to that of 2,4,4′-trichloro-2′-hydroxydiphenyl ether in the practical test. The alleged natural deodorant principle does not necessarily depend on an antimicrobial effect in the sense of killing bacteria (bactericidal effect). Metabolic deactivation and growth inhibition alone (bacteriostatic effect) should also give satisfactory results.

ECOCOMPATIBILITY

HGQ and its individual components and 2,4,4′-trichloro-2′-hydroxydiphenyl ether and 2,2′-methylenebis(6-bromo-4-chlorophenol) were tested for their biodegradability. The

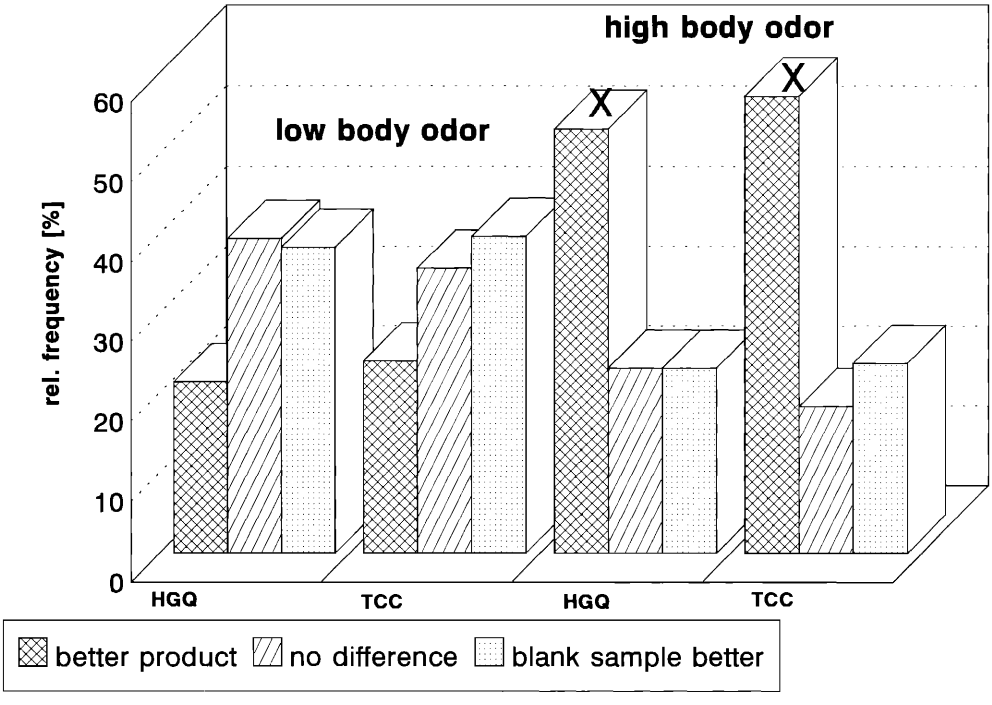

Figure 2. Comparison of the deodorant effect of soaps containing either a natural active substance mixture (HGQ) or TCC (0.26%), solubilized with nonylphenolethoxylate 9 (24) (x = statistically significant difference).

Table II
Sniff Test: Sniff Potential Assessment Scale

0	Fresh, pleasant odor (faint scent of perfume, conditioner)
1	Fresh, pleasant odor
2	Faint musty odor
3	Unpleasant, distinctly musty odor
4	Unpleasant, faint odor of sweat
5	Unpleasant, strong odor of sweat
P	Strong perfume masking everything else

The sniff potential is assessed by three experts.

latter two substances were widespread, especially in the deodorant sector, and served as the standards (4).

Biological degradability is tested by measuring the amount of oxygen required for oxidation. A distinction is made between the chemical oxygen demand (COD) and the biological oxygen demand (BOD).

Chemical oxygen demand is a constant and corresponds to the amount of chromium(VI) compounds—expressed as oxygen relative to a 1-liter sample of water—consumed under defined conditions by the reducing components of contaminated water.

Biological oxygen demand is defined as the amount of oxygen required by microorganisms from activated sludge basins for oxidative degradation of the organic substances

Table III
Results of a Comparison of Three Spray Preparations

Product A: contains 2,4,4′-trichloro-2′-hydroxydiphenyl ether (0.26%, solubilized)	
Product B: contains HGQ (0.3%)	
Product C: placebo	
Sniff test	
A><B	6 h: no difference in sniff potential
A><B	12 h: no difference in sniff potential
A><B	24 h: no difference in sniff potential
Self-assessment	
Evaluation of the questionnaire revealed that the subjects reported no preference for either of the two products.	
Sniff test	
B><C	3 h: B significantly better than C in sniff potential
B><C	12 h: B significantly better than C in sniff potential
B><C	24 h: B significantly better than C in sniff potential
Self-assessment	
Evaluation of the questionnaire revealed: After 3 h and 12 h the subjects clearly preferred B. After 24 h the subjects reported that B seemed more effective.	

contained in a 1-liter water sample under given test conditions (time, temperature, etc.) (23).

The BOD subscript indicates the test period in days. The BOD_5/COD ratio is often formed, which represents the relative biological degradability.

The results of these types of test are naturally subject to variations. The information obtained on the degradation behavior, i.e., return of the component substance to the natural cycle, does, however, allow an estimation of the impact on the environment.

Two grams of each product were mechanically shaken for 24 hours, the undissolved fraction separated, and the chemical and biochemical oxygen demand of the dissolved components determined. For this reason, the results are not related to the product itself but to the aqueous solution prepared as described. The data permit estimation of the degradability on the basis of the BOD_5/COD ratio (4).

Table IV shows that the soluble fraction of 2,2′-methylenebis(6-bromo-4-chlorophenol) has a very poor biological degradability. For 2,4,4′trichloro-2′-hydroxydiphenyl ether there is apparently a total inhibition of degradation by the microbiocidal effect.

The natural product active substances named above have an acceptable degree of degradability. The degradation products are only CO_2 and H_2O, as HGQ does not contain any nitrogen. (Also see Figure 3.) Furthermore, this finding indicates that although the synergistically acting HGQ mixture has a selective bactericidal effect, it is degraded naturally after use, which is not true of the first deodorant generation.

DISCUSSION

A major component of odor production is eliminated by growth inhibition of coryneform bacteria. It seems probable that the coryneform bacteria do not themselves produce

Table IV
Test Results

Sample name	Chemical oxygen demand COD (mg/l)	Biochemical oxygen demand BOD_5 (mg/l)	BOD_5/COD	%
Glyceryl monolaurate (H)	1755	1069	0.61	61
Farnesol (G)	483	217	0.45	45
Phenoxyethanol (Q)	3205	975	0.30	30
HGQ	2880	728	0.25	25
2,4,4′-Trichloro-2′-hydroxydiphenyl ether	453	<5	—	0
2,2′-Methylenebis-(6-bromo-4-chlorophenol)	778	15	0.02	2

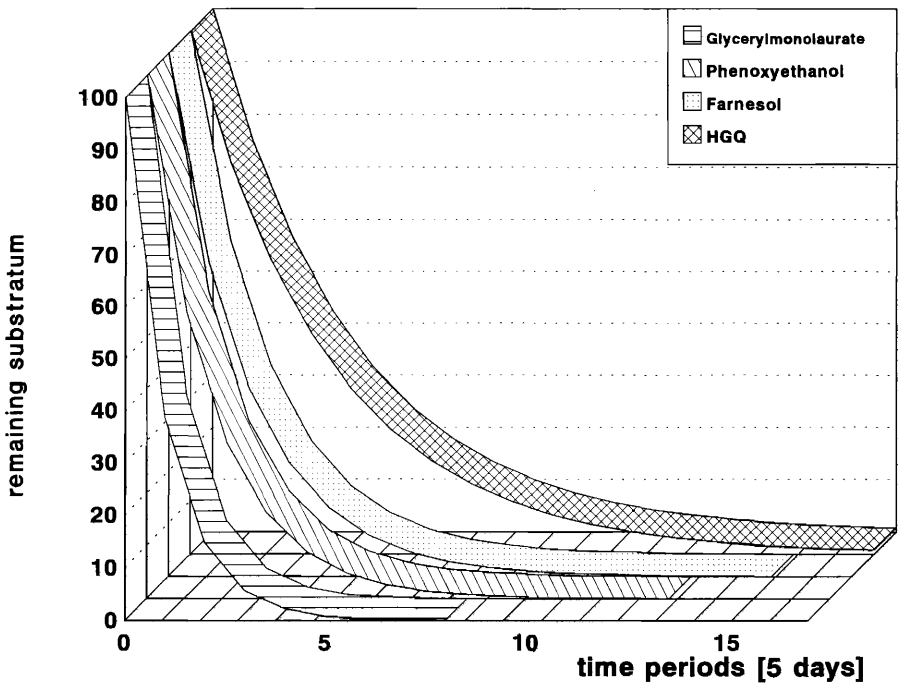

Figure 3. Biological degradability of HGQ and its ingredients (computed from BOD_5/COD).

short-chain fatty acids (18) but that their enzymes release these compounds from apocrine sweat or physiological skin surface lipids.

Apparently only coryneform bacteria are capable of releasing odorous substances such as short-chain fatty acids (19) or steroids. It is remarkable that the bacteria population of the axillae is distinguished by two types of flora. There is a coccal dominance and a corynebacteria dominance (at least 75% of all aerobic bacteria are of the coryneform type). The population density of the coryneform flora is six times that of the coccal dominance (11).

In an application concentration of 0.3% to 0.6%, HGQ acts as an effective deodorant

by reliably inhibiting the growth of coryneform bacteria, the main producers of odorous substances from underarm sweat and skin surface lipids. In addition, it is composed of natural products and has shown no signs of sensitization, i.e., of allergic contact eczema (3).

HGQ and its individual natural active substances are biologically degradable to an acceptable degree. Only CO_2 and H_2O are formed, since there is no nitrogen contained in HGQ.

Furthermore, this finding indicates that although the synergistically acting HGQ mixture has a selective bactericidal action, it is degraded naturally after use, which is not true of the first generation of deodorants.

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