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PLANT HORMONES IN FUNGI AND BACTERIA FROM MALTING BARLEY

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40 Fungi and 16 strains of bacteria, isolated from the grains of three cultivars of malting-grade barley (Kymppi, Pokko and Kustaa, of 1990 harvest), were screened for the production of the plant hormones gibberellic acid (GA_3, abscisic acid (ABA) and indole-3-acetic acid (IAA). Four fungal strains were found capable of GA_3 production and four of ABA production. IAA production was common among both fungi (58% of strains active) and bacteria (88% of strains active). To get an estimate of the physiological significance of the presence of plant hormone producing microbes, the plant hormone production per microbial unit in the liquid growth media of the cultured organisms was weighed against the microbial counts and the endogenous hormone concentrations of barley grains. It was concluded that bacterial IAA production could be of significance in imbibed grains. This presupposes, however, that the conditions be ideal for the propagation of the active species and also, for the production of IAA by those same species and lastly, that similar production occurs *in vivo* as well as *in vitro*. Microbial GA_3 and ABA production, on the other hand, were estimated to occur in negligent amounts.

Key Words: *Malting barley, microorganisms, hormones, gibberellic acid, abscisic acid, indole-3-acetic acid*

INTRODUCTION

Essentially all developmental and dynamic processes of plant life are directed through the modification of hormonal concentration gradients and many of the events that are under the control of endogenously produced hormones can also be affected by applying the effective hormones alone, or in concert with other hormones externally to plant organs. As diverse events as flowering, transpiration, dormancy and germination of seeds, senescence and abscission of leaves or of other organs, cold acclimation, and tropistic responses, can be affected by the application of plant hormones to plants[14]. Consequently, these same processes can in some cases be altered by the introduction of a microbe that produces sufficient quantities of a plant hormone and that is able to supply the plant with the hormone[24,26,41,47,48,52], why it is particularly interesting to note that plant hormone production in microbes is of such wide spread occurrence[24,25,42,46]. Since each seed of a plant can be treated as a separate entity, limited in size and affected only by the composition and physical parameters of its immediate surroundings, seeds have offered a good object when considering the utilization of purified natural or synthetic plant hormones. Interest has been focused on the ability of the seed-borne bacteria and fungi of malting barley to produce plant hormones that might affect the germination or the enzymatic capacity of hosting seeds. GA_3, ABA and IAA would seem to offer promise in this context, not omitting cytokinins and ethylene.

Barley grains, like the grains of other cereals, are exceptionally rich sources of microbes. A wide range of bacteria, yeasts and filamentous fungi colonize the surface and the husk—particularly the area between the husk and pericarp—of mature barley grains both in the field and during storage[6,7,16,27]. When barley grains are imbibed, there is a surge of GA_3 and ABA during the early stages of germination[22,30,60,61], which coincides with a rapid increase in the microbial counts of the grains, especially bacteria[7,16,27]. Later on, when the radicle and plumule have emerged, a build up of IAA can be expected[11,61]. This led the question, what is the incidence of plant hormone producing microbes among the microflora of mature malting-grade barley grains, and could these possibly account for some of the described increase in GA_3, ABA and IAA?

Growth of roots and acrospires during the germination of barley grain requires utilizable food reserves that are released from the breakdown of the starchy endosperm. It is well established that GA_3 released from the embryo following imbibition is primarily responsible for the production of endosperm hydrolyzing enzymes in barley aleurone cells[20]. Secretion of α-amylases and other hydrolyses from detached aleurone layers is greatly enhanced by exogenously applied GA_3 as well[10,23,44,51,55] and this is the reason why GA_3 is the most widely used additive in malting, when permitted by customers and local legislation[37]. Auxins, mainly IAA, have been noted to support the action of GA_3[34,35,45]. When isolated aleurone layers of barley are treated with ABA, a suppression in the synthesis of GA_3 inducible enzymes can be seen. Also, ABA enhances the expression of several ABA-specific genes in barley aleurone layers, including a gene for an α-amylase inhibitor[20]. The overall effect of ABA has been suggested to be a slow endosperm mobilization in response to environmental stresses[19]. The growth inhibiting effect of ABA has been utilized to reduce malting losses caused by the enhanced growth of roots and acrospires, caused by GA_3 addition[60]. The implications of microbial GA_3, ABA or IAA production on grains in *in vivo* circumstances could thus include the stimulation of germination and enzymatic activity (GA_3), maintenance of dormancy and inhibition of enzymatic activity as well as germination (ABA) and, depending on the amounts released, the inhibition or stimulation of germination alongside with a stimulation in enzymatic activity (IAA)[15,34,35,37,60]. Successful malting, on the other hand, requires a favourable balance of GA_3, ABA and IAA concentrations, which is why all the factors affecting the concentrations of these hormones, including microbial activity, are of importance to the maltster.

During the cause of the present study, the bacteria, yeasts and moulds were isolated from three cultivars of barley and screened for the GA_3, ABA and IAA producing ability in enriched, pure cultures. Microbial counts in grains and in

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Abbreviations: ABA, abscisic acid; cfu, colony forming unit; GA_3, gibberellic acid; GC-MS/SIM, gas chromatography-selected ion monitoring-mass spectrometry; IAA, indole-3-acetic acid.

culture media of species together with the hormone content of grains and of microbial culture media were used in estimating the potential influence of microbial GA_3, ABA and IAA production on the grains hosting the microbes.

Materials and Methods

Sample composition

The method was applied to barley (*Hordeum vulgare* L.) grains as well as to liquid growth media of fungi and bacteria, isolated from the grains.

Dry (moisture content *ca.* 8wt%), malting-grade grains of the cultivars Kymppi, Kustaa and Pokko were used. These cultivars are among the four most important barley varieties used by Finnish malt producers (cv Kilta being the fourth). The grains were of 1990 harvest. Parallel cultivations of fungi and bacteria were prepared in each case and parallel samples of grains were subjected to analysis as well.

Isolation and cultivation of fungi and bacteria

Fungi, other than *Fusarom*, and bacteria were isolated from spread plates prepared from milled grains suspended in physiological salt solution. Potato dextrose agar (PDA, Biokar Diagnostics), containing 0.5 g/litre ampicillin, was used in isolating the fungi and Plate-count agar (Merck) with 0.01 g/litre cycloheximide for isolation of bacteria. Fungi were inoculated in both daylight and under near-UV radiation from a black light (Philips, TLD 36W/08) with a 12 h light–dark cycle to induce growth. To distinguish isolates, fungi were grown on Malt extract- (Merck), Czapek-Dox- (Oxoid) and Wort (Difco) agars, as well as on PDA, in both darkness and light. *Fusarium* spp. fail to show on spread plates and were therefore isolated by direct plating of kernels on Modified Czapek-Dox agar containing iprodione and dichloran (CZID media) according to the method described by Abildgren *et al.*[1]. Plated kernels were incubated under a daylight lamp (Sylvania, 36W Activa 172).

Before inoculation of the plant hormone production medium, fungi were grown for sporulation for 24 h at 27°C in a medium containing 30 g/litre glucose, 7.1 g/litre ammonium tartrate, 5 g/litre $MgSO_4 \times 7H_2O$, 2.7 g/litre $(NH_4)_2SO_4$ and 10^3 µl/litre of mineral solution. $5{*}10^3$ µl of physiological salt solution was applied to fungal plates and the surface was scraped, after which the suspension was used to inoculate the sporulation medium. For the purpose of enrichment of plant hormones, fungi were grown for 9 days at 27°C under carbon limited conditions[5], using again glucose (40 g/litre) as sole carbon and energy source and ammonium tartrate (9.5 g/litre) as the nitrogen source. This growth medium also contained 2.0 g/litre KH_2PO_4, 0.6 g/litre K_2SO_4, 0.2 g/litre $MgSO_4 \times 7H_2O$ and 10^3 µl/litre of mineral solution. Erlenmeyer shake flasks containing 0.05 litres of medium and shaken at 190 rpm in darkness were used in both the sporulation and production media. The pH of the production medium was set to 5.8 as opposed to pH 7 in the sporulation medium and it was inoculated with 10^3 µl of sporulation medium. The mineral solution contained 1.0 g/litre $FeSO_4 \times 7H_2O$, 0.15 g/litre $CuSO_4 \times 5H_2O$, 1.0 g/litre $ZnSO_4 \times 5H_2O$, 0.1 g/litre $MnSO_4 \times 7H_2O$, 0.1 g/litre K_2MnO_4 and 3.0 g/litre Na_2EDTA.

Bacteria were grown in darkness for 12 days at 27°C in Erlenmeyer shake flasks containing 0.1 litres each of a medium modified from the medium used by Brown[8]. This medium contained 10.0 g/litre sucrose, 0.5 g/litre KNO_3, 0.5 g/litre yeast-extract (Difco), 5×10^{-3} litres of a trace element solution A (0.1 mg/µl K_2HPO_4 and 0.1 mg/µl KH_2PO_4) and 5×10^{-3} litres of trace element solution B (0.04 mg/µl $MgSO_4 \times 7H_2O$, 0.002 mg/µl NaCl, 0.002 mg/µl $FeSO_4 \times 7H_2O$ and 0.002 mg/µl $MnSO_4 \times 4H_2O$). To inoculate the medium, bacterial culture was applied to $2{*}10^3$ µl of fysiological salt solution and 200 µl of the suspension was used as inocula.

Characterization of strains

Fungal isolates were identified at the Centraalbureau voor Schimmelcultures in Baarn. Isolates belonging to the same species were treated separately if having been isolated from different cultivars. Bacterial isolates were identified at the Technical Research Centre of Finland (Espoo), using ID 32 GN, API 50 CHE, ID 32 E and API 50 CHB (bioMérieux) strips, as well as gas chromatographic data on the lipid composition of the bacterial cell walls. All bacterial strains have been deposited at the culture collection of the Technical Research Centre of Finland.

Sample preparation

Fungal mycelium was separated from growth media by filtration (Whatman No. 3 paper). The growth media were retained and the mycelia washed with distilled water and frozen (–60°C), before lyophilization 48 h (Christ Alpha 2-4). Yeast and bacterial suspensions were separated from the growth media by centrifugation (2000×g, 5°C, 30 min) and the supernatants were retained. The growth media were stored at +5°C, before extraction and derivatization of plant hormones.

Prior to analysis, barley grains were milled (Frithsch Pulverisette 14, sieve size 0.5 mm) for 30 s and parallel samples of 100 g were weighed for the analysis.

Extraction of plant hormones, derivation of samples and GC-MS/SIM analysis

Quantitation of GA_3, ABA and IAA were done according to Tuomi and Rosenqvist[58]. The method of quantitation includes purification by solvent partitioning and thin layer chromatography, preparation of trimethylsilyl derivates with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and analysis by gas chromatography-mass spectrometry (GC-MS), using selected-ion monitoring (SIM).

The inherent error limits and the recoveries of the analysis procedure were measured by performing a double sided students-T test with a 95% confidence interval on the deviation from the mean of 15 GA_3, ABA and IAA standards. The standards were subjected to the same treatment as the samples and the recoveries so obtained were 47% (GA_3), 79% (ABA) and 21% (IAA), whereas the error limits were 21% (GA_3), 42% (ABA) and 51% (IAA).

Enumeration of fungi and bacteria in grains and in culture media

Viable counts in grains were calculated from dilution plates. Cell numbers in the culture media of yeasts and bacteria were calculated using the Hawksey glass counting chamber (A 70 Helber, Hawksley, Ltd.). Cell numbers in the culture media of moulds could not be acquired due to their mode of growth (as pellets). The biological significance of hormone production in moulds was therefore estimated based on the dry weight of the cell mass of cultivated moulds as compared to the dry weight of yeasts, for which a cell number was available.

Quantitative estimates of microbial plant hormone production in grains

By multiplying the hormone contents in the culture media of strains (µg/litre) with the cell numbers in the media (cells/litre), estimates of the hormone producing capacity of the strains (µg/cell) were obtained. This, together with the viable counts of the strains in barley (cells/kg), gave an estimate of the hormone producing potentials of the strains (µg/cell)—or, in other words, of the maximum amount of hormone added by the strains to the hormone pool of the grains. By comparing this estimate with the endogenous hormone contents of the grains (µg/kg), it was possible to appraise whether the microbial population, at its best, could contribute significantly to the GA_3, ABA or IAA pool of the grains.

TABLE I. Percentage of grains contaminated with *Fusarium*

Fusarium sp.	Barley cultivar	Grains contaminated (%)
Ky.Fu01	Kymppi -90	1
Ky.Fu02	Kymppi -90	3
Ky.Fu03	Kymppi -90	1
Ky.Fu04	Kymppi -90	2
Ky.Fu06	Kymppi -90	1
Kus.Fu01	Kustaa -90	2

RESULTS

The barley cultivars had been grown and stored at different locations and partly due to this, there were notable differences in the spectra of microbial species isolated, as well as in the frequency of individual species (Fig. 1(a),(b), Table I). Cv. Kymppi had the richest spectra of field fungi such as *Fusarom* (5 spp.), *Alternaria, Cladosporium* and *Epicoccum*, whereas xerophilic storage fungi, namely *Aspergillus* and *Penicillium*, were prominent in both Kymppi and Kustaa. Cv. Pokko contained exceptionally few fungi, while Kymppi and Kustaa both showed total cfu:s of the order 10^3 (Fig. 1(a)). Cv. Kymppi was clearly richest in bacteria as well, but there were less variation in the total cfu:s of bacteria as well as in the incidence of bacterial species (Fig. 1(b)). Judging from the microbial counts and -spectra of the cultivars, all samples were representative of healthy barley, well suited for malting purposes.

In what follows, the GA_3, ABA and IAA production of isolated species are related. The moulds, which have a different mode of growth than the yeasts and the bacteria, are treated

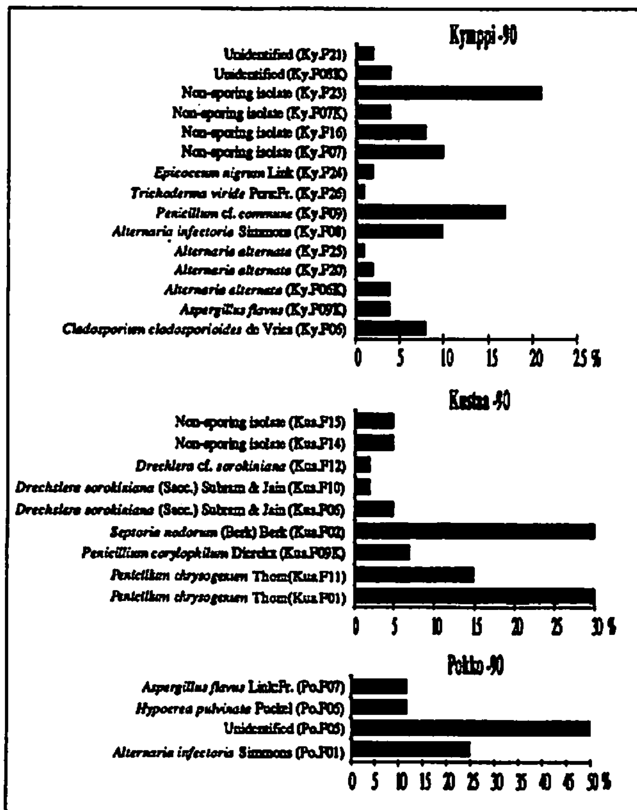

FIG. 1(a). The cfu:s (colony forming units) of the mould isolates expressed as percentages of the total cfu:s of the moulds in the cultivars. *Fusarium* spp. are omitted. The total cfu:s were as follows: 1.5×10^3 (cv Kymppi), 1×10^3 (cv Kustaa) and 4×10^2 (cv Pokko).

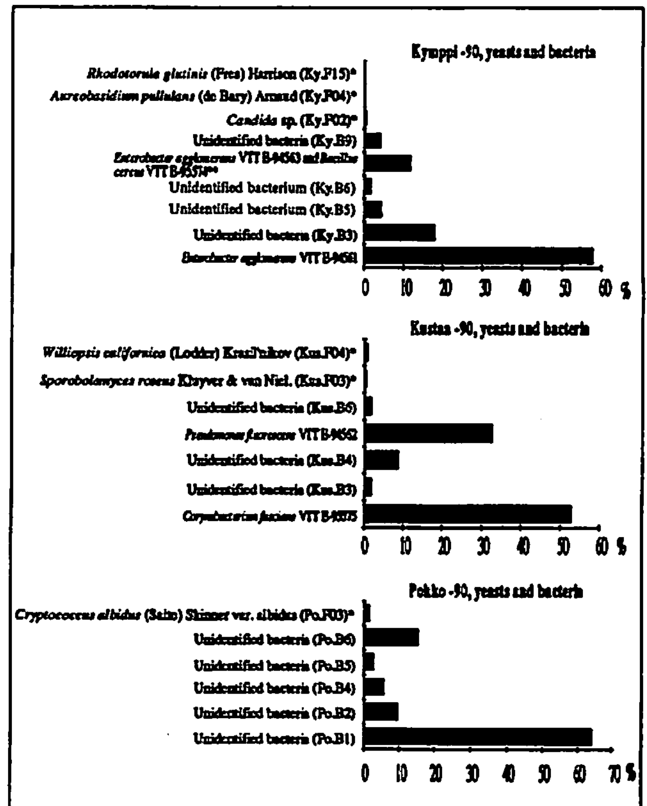

FIG. 1(b). The cfu:s of the yeasts (marked with an asterix*) and the bacteria, expressed as percentages of the combined total cfu of the yeasts and the bacteria in the cultivars. The total cfu:s (yeasts + bacteria) were as follows: $4.4 \times 10^3 + 6 \times 10^5$ (cv Kymppi), $2.5 \times 10^3 + 1.4 \times 10^4$ (cv Kustaa) and $5 \times 10^2 + 3.1 \times 10^4$ (cv Pokko). **Mixed culture.

separately. The means of the endogenous hormone contents of the cultivars, which the quantitative estimations are based on, were 0.774 ± 0.163 µg/kg fresh wt (GA_3), 12.9 ± 5.4 µg/kg fresh wt (ABA) and 195 ± 99 µg/kg fresh wt (IAA). It is known from the literature that the GA_3, ABA and IAA content of dry, mature barley kernels are much lower than in developing, germinating or in germinated grains[13,22,32]. Moreover, the contents of all three hormones vary from cultivar to cultivar so the reported endogenous GA_3, ABA and IAA contents of barley grains hold true for this specific case only. It can however be stated that even though there are notable differences in the hormone contents of dry kernels, and especially in dormant versus non-dormant kernels, the GA_3, ABA and IAA contents of barley seem to be of the same magnitudes as those reported here[13,32].

Hormone production in moulds

Of the 34 moulds assayed, only four were established as GA_3 producers and three as ABA producers (Table II). Most gibberellin producing fungal strains known today are species of *Gibberella* or *Fusarium*[36] (which is the imperfect or anamorphic form of *Gibberella*), but much to our surprise, the GA_3 and ABA producers did not include any *Fusarium* spp. (Tables I and II). Among the GA_3 producers was one non-sporing species, as well as one species of *Alternaria, Penicillium* and *Aspergillus*, respectively. The ABA producing mould isolates belongs to the *Alternaria, Aspergillus* and "non-sporing" groups. The *Alternaria (A. alternata)* and *Aspergillus (A. flavus)* isolates were capable of both GA_3 and ABA production (Table II). These are important findings in themselves, since GA_3 and ABA producing fungi are very scarce in the literature[24,46] and apart from the studies of Crocoll *et al.*[12],

TABLE II. GA_3 and ABA producing strains. The yields are expressed as μg of GA_3 and ABA per litre of culture medium

STRAIN	GA_3 (μg/litre)	ABA (μg/litre)
Alternaria alternata (Ky.F25)	1.36 ± 0.29	10.20 ± 4.28
Non-sporing isolate (Ky.F07)	17.73 ± 3.72	0
Non-sporing isolate (Ky.F23)	0	17.37 ± 7.30
Penicillum chrysogenum Thom (Kus.F01)	5.60 ± 1.17	0
Williopsis californica (Lodder) Krasil'nikow (KusF04)*	0	43.10 ± 18.10
Aspergilus flavus Link:Fr. (Po.F07)	1.32 ± 0.28	60.43 ± 25.38

*Yeast.

Dörffling and Petersen[17] and Rademacher[47] it does not appear that microbes have been screened for gibberellin or ABA production and where results have been verified by GC-MS/SIM. As to the contribution of microbial GA_3 or ABA to the hormonal pool of grains, the estimated maximum amounts would not seem to be high enough to be significant.

IAA production on the other hand was more frequent, 20 of the 34 strains, or 59% being productive (Fig. 2). Of the IAA producing fungi, six were *Fusarium* spp., as all *Fusarom* produced IAA (Table I, Fig. 2). Fungal counts in grains did not, however, give any indication that this production, either, would bear much significance when considering the IAA pool of the grains. The IAA producing incidence of the moulds is shown in Figure 2, which shows that there were no notable differences in the amounts of IAA produced. The most potent producer was a non-sporing species that also produced GA_3 but not ABA (Fig. 2, Table II).

Hormone production in yeasts

None of the yeasts produced GA_3 and ABA was produced by one species of yeast (*Williopsis californica* (Lodder) Krasil'nikow) only (Table II). In the yeasts, as in the moulds, IAA production was more common, as three out of six yeasts produced IAA. Yeasts usually constitute a minority of the microbial population of barley grain[16,27], however, and they are not present in numbers high enough for yeast-derived IAA to be of significance with regard to the development of the grains (Fig. 3). It would, however, be interesting to probe if the relatively high amount of IAA released by a species of *Candida* (Fig. 3) is a common feature of these organisms.

Hormone production in bacteria

The bacteria produced neither ABA nor GA_3 but they were ardent producers of IAA, 14 isolates of 16 (88%) being active. The bacterial count in dry grains was, however, once again insufficient for the bacterial IAA to be perceived as a significant factor in determining the total IAA pool of grains (Fig. 3). One of the chief purposes for drying grains prior to storage, which precedes processing, is to prevent microbes from flourishing. This is achieved at water contents approaching 12wt%[6]. When grains are wetted, microbial counts, especially the number of bacteria, invariably start to increase. In the steeping of malting barley, for instance, the counts of mesophilic bacteria have been reported to increase up to two orders of magnitude[16] and during the germination phase, the bacterial counts peak at a level of up to three orders of magnitude higher than in dry barley grains[16,27]. In our own steeping experiment (results not shown), in which we used cv Kymppi, the bacterial count varied from 10^5 cfu/g fresh wt at the onset, to a maximum of 10^8 cfu/g fresh wt, some of the bacteria clearly being washed out during water-changes. Bacterial counts in wetted barley grains thus reach levels that could be conceived as sufficient to ensure that IAA is produced by grain-borne bacteria in amounts that do to a significant extent contribute to the total IAA pool of wetted grains (Fig. 3). The estimated additional IAA would seem to vary in amount mainly with the difference in bacterial counts of

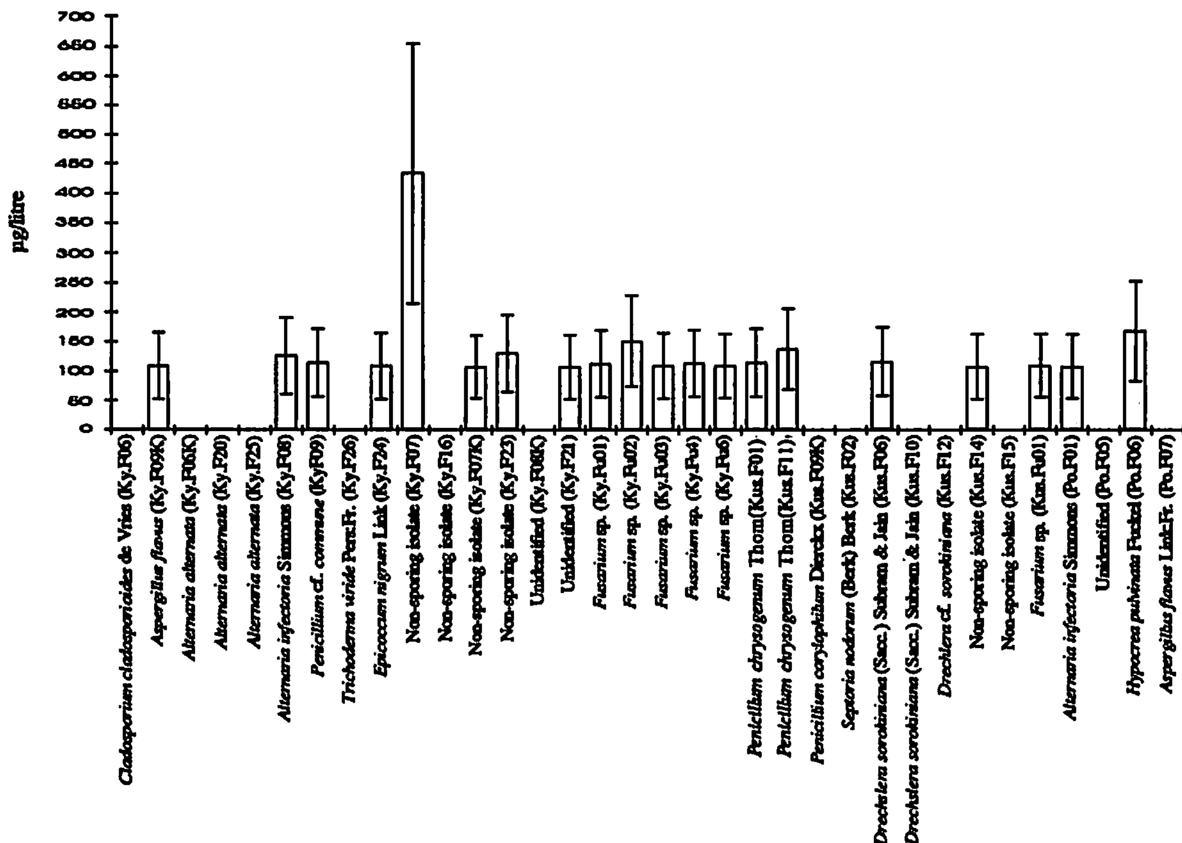

FIG. 2. IAA production of mould isolates, expressed as μg of IAA per litre of culture medium. (I) = 95% confidence limit.

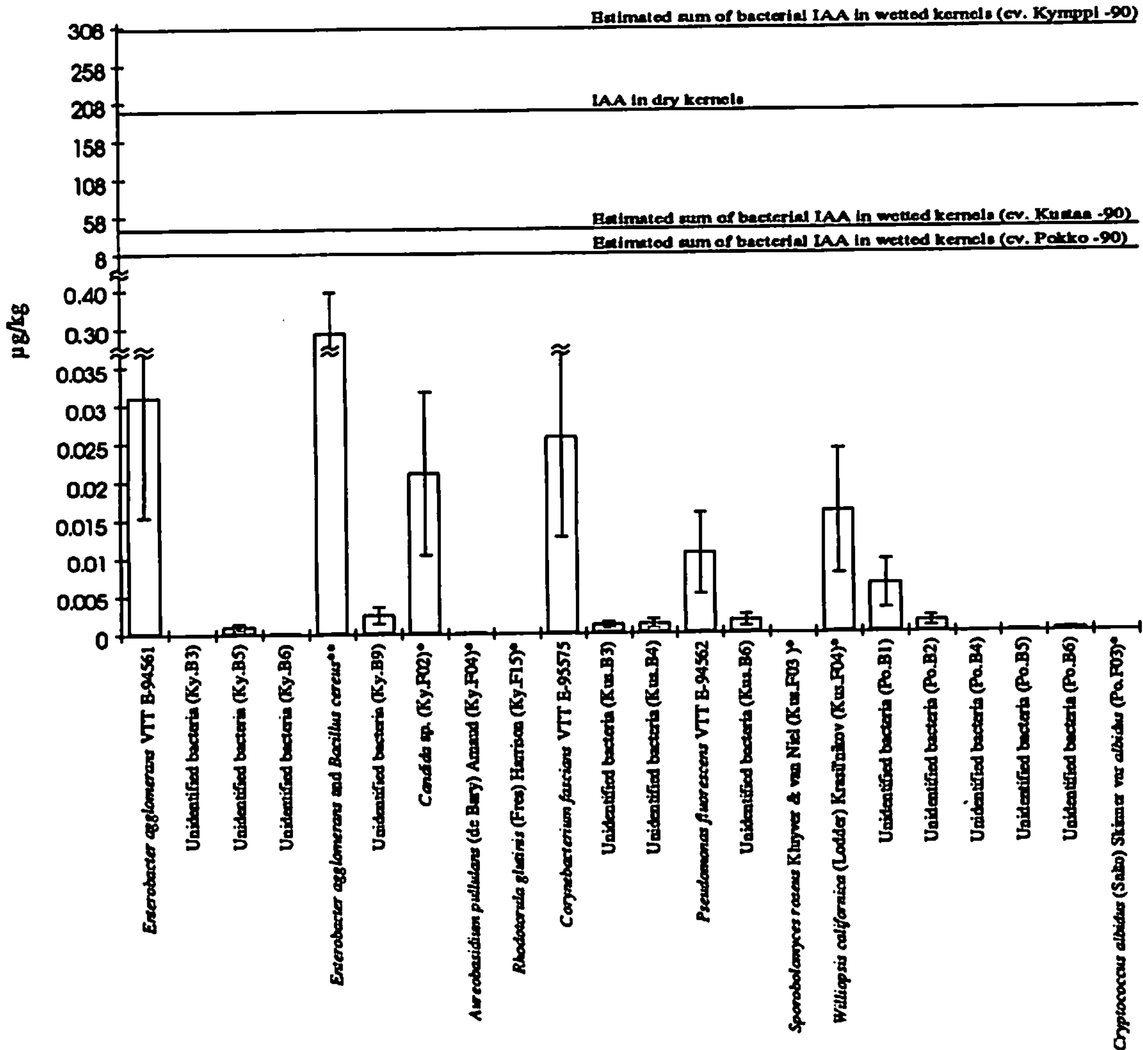

FIG. 3. IAA production in yeasts (marked with an asterix*) and bacteria, expressed as µg IAA per kg of barley grain. (I) = 95% confidence limit. **Mixed culture.

different cultivars. This is illustrated by the fact that the IAA producing ability of bacterial isolates were similar among the cultivars (Fig. 3).

To get further verification of this phenomenon, the most potent IAA producers among the bacteria were identified down to species level (Fig. 1b, 3). Interestingly, both *Enterobacter agglomerans* (more recent classification *Pantoea agglomerans*) and *Pseudomonas fluorescens* have previously been singled out as producing large quantities of auxins in studies dealing with the production of IAA, or compounds exerting auxin-like effects, by plant-associated bacteria[26,41,52,31]. Effects that have been attributed to this production include stimulated root hair production, overgrowth formation, inhibition of root elongation and yield increases[26,41,52]. Also—and more importantly from our point of view—both species are among the bacteria most commonly associated with barley in the field[28] and both are known to proliferate during malting[27]. Noteworthy is also that the count of *Bacillus* species has been found to increase significantly upon malting[16,27]. Only *Corynebacterium fascians* (more recent classification *Rhodococcus fascians*) then, is not normally sited as among the bacterial flora of steeped or germinated barley and, all in all, it is safe to say that the normal, indigenous bacterial flora of barley contains strains producing and excreting IAA.

DISCUSSION

Since the early findings of Nielsen[43], auxin production has been established as common among fungi as well as bacteria. IAA is the principal auxin in higher plants and IAA production has been established in phyllophane fungi[9,56] as well as in phylloplane and epiphytic bacteria[2,40]. It is common among rhizosphere fungi[3,18,54], including ectomycorrhizal fungi, as well as among rhizosphere bacteria[26,41,57]. IAA production has been registered in strains living in such diverse habitats as in the gut of termites[50] and in aquatic plants[21], and it can be concluded that microorganisms from most, if not all habitats that influence the behaviour of plants are known to produce IAA. Not all microorganisms produce IAA, but the percentage of producing strains is greatly increased when tryptophan, which is the immediate precursor of IAA production in many microbes[39], is supplied to the growth media[59].

Pathogenicity is frequently accompanied by great increases in auxins, particularly IAA, but even though it has been proven that plants can be supplied with this hormone by microbes[38], it is usually difficult or impossible to determine whether the excess auxin, resulting from infection with a pathogen, is made by the host as a reaction to infection or by the pathogen, or if it is due to loss of auxinase activity in the host. Increased IAA concentration may be an important factor

in the infection of plants by pathogens. It could act by softening cell walls and inhibiting secondary wall formation, thus improving conditions for the growth of the pathogen[53]. It may also be partly responsible for the increased translocation of solutes, since translocation is frequently known to be directed towards the source of auxin production[4]. Auxin production by saprophytic microorganisms has also been linked to the promotion of growth of host plants and has been suggested to contribute to the potency of biological fertilizers[29,41].

Cuticularized layers and the lignified walls of the surface layers of the grain offer effective protection against penetration of microorganisms. Microbial growth appears to extend to the testa-nucellar cuticle, which acts as a barrier to further penetration. Invasion of microorganisms is, however, made possible when grains crack during drying or harvesting or when the surface layers soften at the high moisture contents required for germination[6]. Most microbiological evaluations of malt production have dealt with the problems caused by microbial activity. Lowered α-amylase activity[33], inhibition in germination, production of harmful mycotoxins[7], foaming and ropiness, as well as gushing of undesirable flavours in beer[49], are among the problems that have been attributed to the microbial flora of barley. It will most likely prove impossible to rid of the microbial activity in malting. The best alternative could be to find out as much as possible about the microbial influence in the early stages of grain germination and to try and modify the microbial flora so that those strains that are least likely to cause problems—or those that have desired effects—will proliferate. According to the present study, bacterial IAA production is one aspect of the microbial activity that should be considered. In malting trials, IAA has been noted to support the effect of added GA_3[34,35,45]. In one experiment, a stimulation of GA_3 action was noted when IAA was added in concentrations of 0.01 μg/kg to 80 μg/kg barley, and an inhibition when 1500 μg/kg IAA was added[45]. Comparison with the estimate of bacterial IAA production in imbibed barley grains (8–305 μg/kg barley) suggests that the variability in GA_3 response among different cultivars of barley[20] could be due to differences in the amount of IAA produced by the bacterial flora. Also, this further underlines the possibility that bacterial IAA could be a factor of significance in the steeping and germination phases of barley malting, and especially in the malting of abraded barley, since abrasion is known to stimulate the uptake of additional, exogenous IAA[45].

This study supports the view that IAA production among fungi and bacteria alike is ubiquitous. The evidence further strongly indicates that bacterial IAA production does occur in imbibed grains in amounts of biological significance. The media used for growth of bacteria did contain tryptophane (a component of yeast-extract), but was not optimized for IAA production. It would therefore be of interest to probe bacterial IAA production in a medium optimized for this purpose and in a setting that more closely resembles *in vivo* conditions. Apart from supporting the effect of added GA_3 in malting, the effects of bacterial IAA on grains could include the inhibition or the stimulation of germination and a stimulation in enzymatic activity[34,35]. It must be stressed though, that indisputable proof of an effect of bacterial IAA production requires the establishment of production as well as of uptake of produced IAA in *in vivo* circumstances.

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