Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 6 (1): 108-114, 2007
ISSN 1682-3974

Phyllosphere Microflora of *Aloe vera* from New Caledonia

[1]H. Amir, [2]C. Isnard, [3]D. Duhet and [3]P. Cabalion
[1]Laboratoire de Biologie et Physiologie Végétales Appliquées, Université de la Nouvelle-Calédonie, B.P. R4, 98851, Nouméa cedex, New Caledonia
[2]CosmecaL Sarl, BP 4159, 98846 Nouméa cedex, New Caledonia
[3]Institut de Recherche pour le Developpement, BP A5, 98848 Noumea cedex, New Caledonia

Abstract: A quantitative and qualitative study of the phyllosphere microflora of *Aloe vera*, a medicinal plant with healing properties, is reported. Bacteria and yeast densities were relatively high. The bacteria were represented mainly by facultatively anaerobic genera, with a domination of *Enterobacteriaceae* (*Enterobacter*, *Klebsiella*, *Erwinia*). *Pseudomonadaceae* (*Xanthomonas* and *Pseudomonas*) were the second group in terms of population densities. *Lactobacillus*, *Micrococcus* and Streptococci were also observed at low frequencies. The bacterial diversity appeared more important in October than in March. The most common fungal genera were identified. Blended leaf fermentation induced transformations of microbial population structure: *Xanthomonas*, *Pseudomonas* and *Micrococcus* were not detectable, whereas *Enterobacter*, *Klebsiella*, *Lactobacillus* and Streptococci (fermentative bacteria) became more abundant. Yeasts were also highly stimulated in fermented leaves.

Key words: *Aloe vera*, phyllosphere, microflora, enterobacteria, fermentation

INTRODUCTION

Aloe vera (L.) N.L. Burman. (syn: *A. barbadensis* Miller) is a Liliaceae traditionally used all over the world for it's different medicinal properties. In present days, many studies are carried out highlighting various mechanisms of aloe bioeffects, as immunostimulatory and antiviral activity (Djeraba and Quere, 2000; Pugh *et al*., 2001), anti-infection and protection of skin (Esteban *et al*., 2000; Richardson *et al*., 2005), anticancer properties (Pecere *et al*., 2000; Wasserman *et al*., 2002), antitoxic activity (Anshoo *et al*., 2005; Gupta and Flora, 2005) and hypoglycaemic activity (Okyar *et al*., 2001; Can *et al*., 2004). New active molecules are regularly identified (Saxena and Chourasia, 2000; Lee *et al*., 2000; Yifang *et al*., 2004).

Several patents have been registered, especially for cosmetic use of aloe leaves. One of these patents (Isnard, 1995) has introduced the use of fermented and distilled leaf extract which was efficient (Hermann, 1992). The standardization of leaf fermentation needs microbiological and ecological studies, but only few have been reported and concerned exclusively hygienic aspects (De Laat *et al*., 1994; Byun *et al*., 1997). The microflora composition of aloe leaves remains unknown.

We therefore performed a quantitative and qualitative study of the phyllosphere heterotrophic microflora of *Aloe vera* and its variation after fermentation of leaves.

MATERIALS AND METHODS

Sites and sampling: Aloe leaves were collected from cultivated fields at three different sites surrounding Noumea (New Caledonia): Boulari, Tamoa and Magenta. The sampling was performed in March (hot and wet season) and in October (temperate season). Except when specified, the plants were over three years old and healthy. In one case, leaves with necroses were collected, in order to compare them with the healthy ones. All leaves were taken on the second row from the periphery of the plant and cut near their base; they were stored at low température (4°C), not more than few hours, before being treated. Each reported value is a mean of 6 replicates corresponding to 6 leaves from different plants.

Microbial population count: As their lower parts were close to the soil, the leaves were rinsed with sterile water to remove the dust, before being finely blended separately in a blender-mixer. Determination of the microbial densities was performed by the dilution-plate count technique.

Corresponding Author: H. Amir, Laboratoire de Biologie et Physiologie Végétales Appliquées, Université de la Nouvelle-Calédonie, B.P. R4, 98851, Noumea cedex, New Caledonia Tel: (687) 26 58 20 Fax: (687) 25 48 29

Ten gram of crushed leaves were suspended in sterile water (90 mL)and shaken vigorously for 20 min after addition of three drops of Tween 80. Appropriate series of 10-fold dilutions were prepared and aliquots of 1 mL were placed in Petri dishes before the medium was added.

For total heterotrophic bacteria, two different media were tested: a classic nutrient agar (peptone: 5 g; yeast extract: 3 g; glucose: 5 g; agar: 15 g: demineralized water: 1000 mL; pH adjusted to 7) enriched with 2% of aloe leaf crush in order to stimulate the growth of aloe dependant bacteria; and Eugon, a fastidious germ medium (trypcase: 15 g; bio-soyase: 5g; sodium chloride: 4 g; sodium sulfite: 0.2 g; L-cystine: 0.7 g; dextrose: 5.5 g; agar: 15 g; demineralized water: 1000 mL; pH adjusted to 7). The first medium was finally chosen for abundance estimates, but both were used for isolation of a wide variety of bacteria. Filter-sterilized actidione (50 mg L^{-1}) was added after sterilization by autoclave to avoid fungal and yeast growth. Total anaerobic bacteria were estimated using the same medium, but the Petri dishes were incubated in anaerobic boxes. Lactic bacteria count was also performed using MRS medium (Lab Lemco: 10 g; peptone: 10 g; yeast extract: 5 g; sodium acetate: 5 g; ammonium citrate: 2 g; potassium phosphate dibasic: 2.4 g.; Tween 80: 1 mL; magnesium sulfate heptahydrate: 0.2 g; manganese sulfate quadrahydrate: 0.05 g; dextrose: 20 g; demineralized water: 1000 mL; agar: 12 g; pH adjusted to 6.5). For this group, the Petri dishes were incubated in a CO_2 enriched atmosphere.

For fungi and yeasts, enriched malt agar was used (malt extract: 10 g; dextrose: 5 g; agar: 20 g; demineralized water: 1000 mL; pH adjusted to 6). 100 mg L^{-1} of filter-sterilized streptomycin and 50 mg L^{-1} of filter-sterilized chloramphenicol were added after autoclaving.

Colonies were counted after 2-4 days incubation at 28°C. Three replicate plates were used for each dilution and for each microbial group.

Microbial identification: For bacterial identification, in a first phase, 250 isolates corresponding to all the colonies of a few petri dishes from Boulari samples and the same number from Tamoa samples, were purified two or three times by streaking on the same media. The following characters were then determined: shape and disposition of cells, Gram, respiration type, mobility, oxidase and catalase. The non-identical strains of each origin were transferred to API galleries. API 20E galleries were used for enterobacteria (completed with lactose fermentation and growth at 10°C), API 20NE for *Pseudomonadaceae*, API 20STREP for Streptococci and API 50CH for *Lactobacillus*. A few other characters were determined especially for Streptococci (growth temperatures, halophilic tenancy, growth range pH). The final identifications were performed according to the Bergey's annual of determinative bacteriology (Williams *et al*., 1989).

In a second phase, when the general composition of the bacterial flora was known, only a few tests were necessary to identify the genera.

Fungal genera were identified after microscopic observation according to different manuals, in particular: Barnett and Hunter (1986), Barron (1977) and Ellis (1993).

Leaf crush fermentation: Over 60 g of blended leaves were put, under sterile atmosphere, into 100 mL sterile bottles. The bottle stoppers were tightened and the samples were incubated at 25 °C for 1 to 5 days. Microbial counts and identifications were then performed with the same techniques as previously described.

RESULTS

Numerical distribution of microorganisms: *Aloe vera* leaves contained 10^5 to 26 10^5 CFU g^{-1} FM (fresh matter) of bacteria (Table 1). Hyphal fungi and yeast populations ranged respectively from 120 to 4, 100 CFU g^{-1} FM and 970 to 4, 300 CFU g^{-1} FM. The leaves collected from the three sites did not support the same quantities of microorganisms: The leaves from Tamoa had the greatest abundance of bacteria and yeasts. Overall, microbial phyllosphere populations were significantly larger in October than in March. The numbers of anaerobic bacteria and lactic bacteria, estimated for leaves from Boulari, in October were respectively: 320, 000 and 18, 000 CFU g^{-1} FM.

Taxonomic distribution of bacteria and fungi: The phyllosphere heterotrophic bacteria of *Aloe vera* isolated in this study represented 5 classical groups: *Enterobacteriaceae*, *Pseudomonadaceae*, *Lactobacillaceae*, *Micrococcaceae* and *Streptococcaceae* (Table 2). The first family was the most frequent (63 to 90% of the total bacteria), with three

Table 1: Densities of different microbial groups in the phyllosphere of *Aloe vera* from 3 different sites (CFU g^{-1} FM)

Sites	Bacteria (X 10^5)	Hyphal fungi (X 10^2)	Yeasts (X 10^2)
Boulari-October	5.6b*	1.5c	15.5b
-March	1.1c	1.2c	9.7c
Tamoa (October)	26.1a	8.3b	43.3a
Magenta (October)	1.2c	41.2a	14.7bc

*Number of anaerobic bacteria: 3.9±1.3 10^5 CFU g^{-1} FM; number of lactic bacteria: 0.18±0.12 10^5 CFU g^{-1} FM. Values followed by the same letter are not significantly different at p = 0.05 (Newman-Keuls test for each column separately)

Table 2: Relative frequencies of the most abundant bacterial genera in the phyllosphere of *Aloe vera* from New Caledonia

	% of the total bacteria*			
	Boulari		Tamoa	
Genera	October	March	October	March
Enterobacteriaceae (total)	62.8	90.0	71.6	88.4
Enterobacter sp.**	42.4	57.6	48.4	49.6
Klebsiella sp.***	19.6	31.2	21.6	38.8
Erwinia sp.	0.8	1.2	1.6	0.0
Pseudomonadaceae (total)	33.2	7.6	23.6	4.0
Xanthomonas sp.	30.8	6.4	21.6	3.6
Pseudomonas sp.	2.4	1.2	2.0	0.4
Lactobacillus brevis	1.2	0.8	2.8	2.4
Micrococcus sp.	0.4	0.0	1.2	0.0
Streptococcus sp.	2.0	1.2	0.8	5.0
Lactococcus lactis				
Unidentified****	0.4	0.4	0.0	0.0

*Over 250 isolates were identified for each site and for each sampling season; **_E. sakazaki_, _E. agglomerans_ and _Enterobacter_ sp., ***_K. oxytoca_ and _K. pneumoniae_, ****very weak growth

genera identified: *Enterobacter*, *Klebsiella* and *Erwinia*. *Pseudomonadaceae* were represented by *Xanthomonas* (7 to 33% of the total bacteria) and *Pseudomonas* (less than 3%). *Lactobacillus, Micrococcus* and Streptococci were uncommon (≤5%).

The anaerobic bacteria obtained in an anoxic atmosphere correspond to Enterobacteria and Streptococci. Strictly anaerobic species were not observed, but facultatively anaerobic bacteria represented 65 to 93% of the total heterotrophic bacteria.

The bacterial flora was more diversified in October than in March and this was observed for both sites, because of the dominance of *Enterobacter* and *Klebsiella* in march.

The hyphal fungal flora (Table 3) was represented by the genera *Penicillium*, *Paecilomyces*, *Fusarium*, *Aspergillus*, *Arthrinium*, *Geotrichum*, *Monodictys*, *Mucor*, *Aureobasidium*, *Cryptococcus*, sterile mycelium and two unidentified yeasts.

Quantitative and qualitative variations of bacterial populations according to the state or the parts of aloe leaves: The number of bacteria in the phyllosphere of aloe was significantly higher on necrotic leaves (Table 4). In this case, the relative frequency of the enterobacteria decreased from over 91 to 59% and *Xanthomonas* and *Lactobacillus* became more frequent. The young leaves contained only 10% of the bacteria found in adult leaves; *Xanthomonas* was absent and replaced by *Lactobacillus* (6.3%).

Quantitative and qualitative variations of bacterial flora of aloe leaf crush after fermentation: After 24 h incubation at 25°C, the microorganisms proliferated greatly on the blended leaves (Table 5). The number of bacteria was multiplied by 600, hyphal fungi by 140 and yeasts by more than 6000. The bacterial population

Table 3: List of fungi isolated from leaves of *Aloe vera* from New Caledonia

Sites	
Boulari	Tamoa
Penicillium sp.*	*Penicillium* sp.
*Fusarium oxysporum**	*Paecilomyces* sp.
F. roseum	*Fusarium oxysporum**
Arthrinium sp*	*F. roseum*
Aspergillus sp.	*Fusarium* sp.
Geotrichum sp.	*Aspergillus* sp.
Monodictys levis	*Geotrichum* sp.
Unidentified (sterile mycelium)	*Mucor* sp.
*Aureobasidium pullulans**	Unidentified (sterile mycelium)
Cryptococcus sp.	*Aureobasidium pullulans**
Unidentified yeast 1*	Unidentified yeasts 1* and 2

*Most frequent fungi

Table 4: Variation of bacterial densities (CFU g^{-1} FM) and percentages of the most frequent bacterial groups depending on the state or the part of *Aloe vera* leaves from Boulari

Samples*	Density (X 10^5)	Dominant groups (number of isolates recovered)	
Healthy adult leaves (3 years)	3.1b	Enterobacteria:	137
		Xanthomonas:	11
		Streptococci:	1
		Lactobacillus:	2
Adult leaves with necroses	15.9a	Enterobacteria:	74
		Xanthomonas:	34
		Lactobacillus:	16
Healthy young leaves		Enterobacteria:	151
(8 months)	0.3c	*Lactobacillus*:	10

*Collected in March; Values followed by the same letter are not significantly different at p = 0.05 (Newman-Keuls Test)

Table 5: Densities of different microbial groups in blended and fermented *Aloe vera* leaves taken from Boulari* (CFU g^{-1} FM)

Days of fermentation	Bacteria (X 10^8)	Hyphal fungi (X 10^4)	Yeasts (X10^5)
1	3.3c	2.10a	101.6b
3	12.4**	1.90a	203.7a
5	20.1a	0.03b	10.5c

*Samples collected in October; **Number of lactic bacteria 3 days after fermentation:1.8×10^8 CFU g^{-1} FM. Values followed by the same letter are not significantly different at p = 0.05 (Newman-Keuls Test for each column separately)

Table 6: Frequencies of the most abundant bacterial genera in fermented blend of *Aloe vera* leaves from Boulari*

Genera	(%) of the total bacteria**
Enterobacteriaceae (total)	87.3
Enterobacter	40.3
Klebsiella	47.0
Erwinia	0.0
Pseudomonadaceae (total)	0.0
Xanthomonas	0.0
Pseudomonas	0.0
Lactobacillus	7.6
Micrococcus	0.0
Streptococcus+Lactococcus	4.2

*Collected in October; **238 isolates identified

continued to increase after 3 and 5 days incubation and reached 20×10^8 CFU g^{-1} FM, but yeasts and fungi stayed at a similar level before
decreasing clearly after 5 days. The density of lactic bacteria, after 3 days incubation, was 1.8 10^8 CFU g^{-1} FM, which represent 14.5% of the bacterial flora.

The equilibrium between bacterial genera was also changed after 3 days fermentation (Table 6). *Erwinia*, *Xanthomonas, Pseudomonas* and *Micrococcus* were not detectable, whereas *Klebsiella*, *Lactobacillus* and Streptococci became relatively more frequent.

DISCUSSION

It is important to emphasize that the techniques used here for counting and isolation of microorganisms do not allow the detection of all the species living in aloe phyllosphere. The media used, even when enriched with aloe crush, are favorable mainly to a part of heterotrophic microbes and some microorganisms might not be detected. As stressed by Yang *et al*., (2001), the microbial phyllosphere populations are more complex than generally reported using classical culture methods. But in this field, all techniques have limits; it seems important to make a choice in relation with the objectives of the study. Thus it is clear that the numbers, the diversity and the proportions of the various genera reported here are relative values and that comparisons should be made carefully, with studies using similar techniques.

Bacterial numbers estimated in the phyllosphere of *Aloe vera* (10^5 to 2×10^6 CFU g^{-1} FM) were similar to those generally reported with the same methods for other plants (Hirano and Upper, 1991; Pati and Chandra, 1993; Babic *et al*., 1996; Yadav *et al*., 2004). It should be noted that aloe leaves are heavy (160-190 g per leaf) because of the water in their pulp, so that the number of microorganisms per leaf was actually very high.

The phyllosphere is known as a highly selective habitat for microorganisms (Lamb and Brown, 1970). Furthermore, *Aloe vera* leaves are very rich in carbohydrates, especially polysaccharides (aloe gel) which represent a suitable source for growth; but it also contains different anti-microbial substances as barbolin, isobarbaloin, aloenin and aloeresin (Gutterman and Chauser-volfson, 2000; Kuzuya *et al*., 2001; Ferro *et al*., 2003). The structure of aloe microbial communities probably is the result of these two opposite effects. In addition, the relatively acid pH (4.2-4.5), constitutes a selective factor. All bacterial genera found in the phyllosphere of aloe have been reported as inhabitants of other leaves (Andrews and Hirano, 1991, Perissol *et al*., 1993, Thompson *et al*., 1993; Yang *et al*., 2001), but their relative frequencies seem to be very distinctive. Indeed, the dominance of Enterobacteria (60 to 90% of the microflora), especially *Enterobacter* and *Klebsiella*, has not been reported for any other plant species and is probably the consequence of the selectivity created by the specific chemical composition of aloe leaves. It must be noticed that different species of enterobacteria, especially in the genera *Enterobacter* and *Klebsiella* has been reported as plant growth promoting bacteria (Haahtela *et al*., 1990; Rönkkö *et al*., 1993; Kämpfer *et al*., 2005). Only 3 genera (*Enterobacter, Klebsiella* and *Xanthomonas*) constitute more than 90% of the bacterial flora. The domination of the phyllosphere microflora by a few genera has been frequently observed (Morris, 1985; Van Outryve *et al*., 1989; Hirano and Upper, 1991). *Erwinia, Pseudomonas and Micrococcus,* common inhabitants of the phyllosphere (Hirano and Upper, 1991), were found here at low percentages (less than 3%).

The most abundant fungal genera were: *Penicillium*, *Fusarium*, *Arthrinium* and *Aureobasidium.* However, very common inhabitants of the phyllosphere, like *Alternaria* and *Cladosporium* (Fokkema, 1991; Sadaka and Ponge, 2003) were not found in aloe leaves.

Microbial numbers in the aloe phyllosphere were higher in October than in March. In New Caledonia, October is a spring period and plants have a better growth in this season. The structure of bacterial communities also changed: *Xanthomonas* was less abundant in March, for the benefit of enterobacteria and the diversity was higher in October. Seasonal variations in the phyllosphere have been frequently reported (Lindow, 1991; Thompson *et al*., 1993; Perissol *et al*., 1993; Legard *et al*., 1994; Yadav *et al*., 2004) and related to the variation of climatic factors such as temperature, moisture and sunlight. A possible explanation for these changes in aloe microflora is that March is a hot month (30-34°C), the leaves under full sun reaching even higher temperatures. In these conditions, some genera would be not competitive and their numbers might fall down. Young leaves contained densities of microorganisms ten times smaller than adult leaves. As stressed by Lindow (1991), plants emerging from soil contain very few epiphytic bacteria and are gradually colonized by specific microbes, so this observation reflects the dynamics of colonization.

The fermentation process occurring in blended aloe leaves is probably similar to the decomposition of senescent leaves in nature. The structure of microbial communities was clearly transformed: the numbers of bacteria and yeasts were over 1000 times higher and the non-fermentative bacteria (*Pseudomonas*, *Xanthomonas*, *Micrococcus*) were not detectable because they could not support oxygen rarefaction. On the contrary, *Klebsiella*, *Enterobacter*, *Lactobacillus* and Streptococci, all capable of growth with a very low oxygen pressure, became more abundant. The loss of hyphal fungi during fermentation supports the hypothesis that conditions became anaerobic as these microbes are usually intolerant to such conditions. Enterobacteriaceae fermentation is generally characterized by the production of several acids as acetic acid, lactic acid, formic acid, succinic acid and ethanol. *Klebsiella* and *Enterobacter* are VP+; this indicate their capability to produce also acetoin and butanediol. *Lactobacillus* and Streptococci produce a lactic fermentation, with production of lactic acid. The aloe crush would have been chemically transformed, with enrichment in different organic acids and rarefaction of carbohydrates inducing the liquefaction of the gel. Indeed, HPLC and gas chromatography analyses (Isnard, Duhet, Cabalion, unpublished data) indicated the presence of some fermentation metabolites, especially acetic acid, ethanol, acetoin and 2,3 butanediol, which confirm the importance of *Enterobacteriaceae*, especially *Klebsiella* and *Enterobacter* (VP+), in aloe fermentation.

This study showed some distinctive characteristics of the phyllosphere microflora of *Aloe vera*, especially the dominance of enterobacteria. The study of leaves fermentation, which revealed quantitative and qualitative transformations of the microflora, will allow a better optimization and stabilization of the fermentation process. Further research will concern especially the identification of metabolites secreted inside the leaf crush during the fermentation.

ACKNOWLEDGMENTS

We want to thank Marie-Pierre Siorat, Eliane Elie-Joly, Atika Amir and Claude Douchement, for their technical support. This research was supported by the Ministère de l'Enseignement et de la Recherche Scientifique of France.

REFERENCES

Andrew, J.H. and S.S. Hirano, 1991. Microbial Ecology of Leaves. Brock/spinger Series, Springer Verlag, London.

Anshoo, G., S. Singh, A.S. Kulkarni, S.C. Pant and R. Vijayaraghavan, 2005. Protective effect of *Aloe vera* L. gel against sulphur mustard-induced systemic toxicity and skin lesions. Ind. J. Pharmacol., 37: 103-110.

Babic, I., S. Roy, A.E. Watada and W.P. Wergin, 1996. Changes in microbial populations on fresh cut spinach. Intl. J. Food Microbiol., 31: 107-119.

Barnett, H.L. and B.B. Hunter, 1986. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company, Mineapolis.

Barron, G.L., 1977. The Genera of Hyphomycetes from Soil. RE Krieger Publishing Company, Huttington.

Byun, M.W., H.S. Yook and O.J. Kwon, 1997. Comparative effects of gamma irradiation and ozone treatment on hygienic quality of aloe powders. Intl. J. Food Sci. Technol., 3: 221-227.

Can, A., N. Akev, N. Ozsoy, S. Bolkent, P.A. Bahriye, R. Yanardag and A. Okyar, 2004. Effect of *Aloe vera* leaf gel and pulp extracts on the liver in type-II diabetic rat models. Biol. Pharm. Bull., 27: 694-698.

De Laat, P.C.A., J.T.W. Verhoeven and J.D. Janse, 1994. Bacterial leaf rot of *Aloe vera* L., caused by *E. chrysanthemi* biovar 3. Eur. J. Plant Pathol., 100: 81-84.

Djeraba, A. and P. Quere, 2000. *In vivo* macrophage activation in chickens with acemannan, a complex carbohydrate extracted from *Aloe vera*. Intl. J. Immunopharmacol., 22: 365-372.

Ellis, M.B., 1993. Dematiaceous Hyphomycetes. CAB International, Oxon.

Esteban, A., J.M. Zapata, L. Casano, M. Martin and B. Sabatier, 2000. Peroxidase activity In *Aloe barbadensis* commercial gel: Probable role in skin protection. Planta Med., 66: 724-727.

Ferro, V.A., F. Bradbury, P. Cameron, E. Shakir, S.R. Rahman and W.H. Stimson, 2003. *In vitro* susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to inner gel of *Aloe barbadensis* Miller. Antimicrob. Agents Chemother., 47: 1137-1139.

Fokkema, N.J., 1991. The phyllosphere as an ecoligically neglected milieu: A Plant Pathologist Point of View. In: Microbial Ecology of Leaves. Andrew, J.H. and S.S. Hirano (Eds.), Springer Verlag, London, pp: 3-18.

Gupta, R. and S.J.S. Flora, 2005. Protective value of *Aloe vera* against some toxic effects of arsenic in rats. PTR Phytother. Res., 19: 23-28.

Gutterman, Y. and E. Chauser-valfson, 2000. The distribution of the phenolic metabolites barbolin, aloeinand aloenin as a peripheral defense strategy in the succulent leaf parts of *Aloe arborescens*. Biochem. Syst. Ecol., 28: 825-838.

Haahtela, K., R. Rönkkö, T. Laasko, P. Williams and T.K. Korhonen, 1990. Root-associated *Enterobacter* and *Klebsiella* in *Poa pratensis*: Characterization of an iron-scavenging system and a substance stimulating hair root production. Mol. Plant-Microbe, 3: 358-365.

Hermann, B., 1992. A propos d'un extrait d'aloès proposé dans le traitement de l'hyperséborrhée du cuir chevelu et de la chute de cheveux. J. Méd. Chir. Esth. Dermatol., 19: 122-131.

Hirano, S.S. and C.D. Upper, 1991. Bacterial Community Dynamics. In Microbial Ecology of Leaves. Andrew, J.H. and S.S. Hirano (Eds.), Springer Verlag, London, pp: 271-294.

Isnard, C., 1995. Aloe Water preparation. U.S. patent N° 5,427,776.

Kämpfer, P., S. Ruppel and R. Remus, 2005. *Enterobacter radicincitans* sp. nov., a plant promoting species of the familly *Enterobacteriaceae*. Syst. Applied Microbiol., 28: 213-221.

Kuzuya, H., I. Tamai, H. Beppu, K. Shimpo and T. Chihara, 2001. Determination of aloenin, barbaloin and isobarbaloin in Aloe species by micellar electrokinetic chromatography. J. Chromatogr. B., 752: 91-97.

Lamb, R.J. and J.F. Brown, 1970. Non parasitic microflora on the surface of living leaves of *P. dilatatum*, *Salix babylonica* and *Eucalyptus stellulata*. Trans. Bri. Mycol. Soc., 55: 383-390.

Lee, K.Y., S.T. Weintraub and Y.P. Byung, 2000. Isolation and identification of a phenolic an tioxidant from *Aloe barbadensis*. Free Rad. Biol. Med., 28: 261-265.

Legard, D.E., M.P. Mcquilken, J.M. Whipps, J.S. Fenlon, T.R. Fermor, I.P. Thompson, M.J. Bailey and J.M. Lynch, 1994. Studies of seasonal changes in the microbial populations on the rhizosphere of spring wheat as a prelude to the release of a genetically modified microrganism. Agric. Eco. Environ., 50: 87-101.

Lindow, S.E., 1991. Determinants of Epiphytic Fitness in Bacteria. In: Microbial Ecology of Leaves. Andrew, J.h. and S.S. Hirano (Eds.). Springer Verlag, London, pp: 295-314.

Morris, C.E., 1985. Diversity of epiphytic bacteria on snap bean leaflets. Ph.D. Thesis, University of Wisconsin, Madison, WI., pp: 210.

Okyar, A., A. Can, N. Akev, G. Baktir and N. Sütlüpinar, 2001. Effect of *Aloe vera* leaves on blood glucose level in type I and II diabetic rat models. Phytother. Res., 15: 157-161.

Pati, B.R. and A.K. Chandra, 1993. Diazotrophic bacterial populations and other associated organisms on the phyllosphere of some crop plants. Zentralbl. Micrkrobiol., 148: 392-402.

Pecere, T., V. Gazzola, C. Mucignat, C. Parolin, F. Dalla Vecchia, A. Cavaggioni, G. Basso, A. Diaspro, B. Salvato, M. Carli and G. Palu, 2000. Aloe-emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. Cancer Res., 60: 2800-2804.

Perissol, C., M. Roux and J. Le Petit, 1993. Succession of bacteria attached to evergreen oak leaf surfaces. Eur. J. Soil Biol., 29: 167-176.

Pugh, N., S.A. Ross, M.A. Elsohly and D.S. Pasco, 2001. Characterization of aloeride, a new high-molecular-weight polysaccharide from *Aloe vera* with potent immunostimulatory activity. J. Agric. Food Chem., 49: 1030-1034.

Richardson, J., J.E. Smith, M. Mcintyre, R. Thomas and K. Pilkington, 2005. *Aloe vera* preventing radiation-induced skin reactions: A systematic literature review. Clin. Oncol., 17: 478-484.

Rönkkö, R., A. Smolender, E.L. Nurmiaho-Lassila and K. Haahtela, 1993. *Frankia* in the rhizosphere of nonhost plants: A comparison with root-associated N2-fixing *Enterobacter* sp., *Klebsiella* sp. and *Pseudomonas* sp. Plant Soil, 153: 85-95.

Sadaka, N. and J.F. Ponge, 2003. Fungal colonization of phyllosphere and litter of *Quercus rotundifolia* Lam. in a holm oak forest (High Atlas, Morocco). Biol. Fertil. Soils, 39: 30-36.

Saxena, V.K. and S. Chourasia, 2000. 7-hydroxy,6,3',4'-trimethoxy isoflavone-5-0-α-L-rhamno-pyranosyl [1-6]-O β-D-glucopyranoside of *Aloe vera*. J. Instit. Chem., 72: 195-198.

Thompson, I.P., M.J. Bailey, J.S. Fenlon, T.R. Fermor, A.K. Lilley, J.M. Lynch, P.J. Mccormack, K.J. Mcquilen, P.B. Painey and J.M. Whipps, 1993. Quantitative and qualitative seasonal changes in the microbial community from the phyllosphere of sugar beet (*Beta vulgaris*). Plant Soil, 150: 177-191.

Van Outryve, M.F., F. Gossele and J. Swings, 1989. The bacterial microflora of witloof chicory (*Cichorum intybus* L. var. *foliusum* Hegi) leaves. Microb. Ecol., 18: 175-186.

Wasserman, L., S. Avigad, E. Beery, J. Nordenberg and E. Fenig, 2002. The effect of aloe emodin on the proliferation of a new Merkel carcinoma cell line. Am. J. Dermatopathol., 24: 17-22.

Williams, S.T., M.E. Sharple and J.G. Holt, 1989. Bergey's Manual of Determinative Bacteriology. Vol. 4, The Williams and Wilkins Co., Baltimore, MD.

Yadav, R.K.P., J.M. Halley, K., Karamanoli, H.I. Constantinidou and D. Vokou, 2004. Bacterial populations on the leaves of Mediterranean plants: Quantitative features and testing of distribution models. Environ. Exp. Bot., 52: 63-77.

Yang, C.H., D.E. Crowley, J. Borneman and N.T. Keen, 2001. Microbial phyllosphere populations are more complex than previously realized. Proc. Nat. Acad. Sci. U.S.A., 98: 3889-3894.

Yifang, Y., W. Hongmei, G., Lei and C. Yi, 2004. Determination of three compounds in *Aloe vera* by capillary electrophoresis. BMC, Biomed. Chromatogr., 18: 112-116.