TAXONOMIC CHARACTERISATION OF ACTINOBACTERIA ISOLATED FROM THE ATMOSPHERE SURROUNDING CHAMOMILE PLANTS

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Abstract. Many different microbes, including actinobacteria thrive in the conditions offered by moisture nutrient materials, which are needed for microbial growth. The actinobacteria isolated and identified from the atmosphere surrounding chamomile plants cultivated at El-Fayoum Governorate, Egypt reached a maximum concentration during harvesting period of chamomile plants. The main components of the air-borne bacteria in the atmosphere of the studied area were Streptomyces, Micrococcus , Rhodococcus, Arthrobacter, Microbacterium, Cellulomonas and Nocardia. These bacteria have an important role in the spreading of allergic diseases among the population during the harvesting of the chamomile plants and indicate a potential risk of occupational respiratory disease.
Key words: *characterisation, actinobacteria, atmosphere, chamomile plant.*

Introduction

The long-term inhalation of organic dusts may cause inflammation of the respiratory tract in reposed agricultural workers as a result of specific reactions [38, 4 and 21]. Mostly, microorganisms associated with organic dusts induce these reactions. These microorganisms have been identified as common causative agents of respiratory disorders due to inhalation of organic dusts, such as allergic alveolitis, asthma and organic dust toxic syndrome [16,19,20,27 and 23]. The harmful effect of allergens and endotoxins may caused by Gram – negative bacteria [8, 19, 24, 30 and 32], actinomycetes [18 and 21] and filamentous fungi [21, 33 and 34].

Grain handling workers (harvesting, threshing, loading, unloading and shuffling) who are associated with heavy exposure to grain dust is regarded as emplyoees in hazard occupation [4, 22, 25, 35, 40, 3 and 5]. Work – related symptoms have been reported in 20-44% of farmers handling grain in England, Poland and Canada [4, 8 and 37] and in 44.8 –89.4% of grain soil workers in Poland, U.S.A., Canada and Hungary [15, 36, 8, 7 and 31].These effects of grain dust are largely due to the action of microorganisms associated with grain and their products which occur in great concentrations in air polluted with grain dust [6, 9 and 17]. Of particular importance are actinobacteria and fungi which develop in incorrectly preserved and overheated grain. It was reported as a cause of allergic alveolitis and other diseases in many countries [21, 19 and 20].

The aim of the present work was to characterise the actinobacteria communities isolated from the atmosphere surrounding the chamomile plant from the beginning of cultivation till harvesting, it to assess the effect of the exposure to grain dust in the populations of the Egyptian farmers engaged at the harvesting of the plant.

Material and Methods

Sampling Site

The samples were taken form El-Fayoum Governorate (~100 km south west of Cairo). It is a residential area cultivated with chamomile plant, temperature ranged from 15-30°C and relative humidity varied between 62-72%.

Sampling methods

Air samples (5 samples collected form the study area, from January to March, 2003) were collected from 11 a.m. to 2 p.m. Each set of samples consisted of 5 Petri dishes (10 cm diameter). The gravitational method [29 and 26] was employed for collection of actinomycetes. Petri plates contained 3% malt extract agar (Difco, Detroit, Mi) were exposed to air for 15 min. The samples were taken at 1.5 m above the ground level from vegetated area. Plates were incubated at 25°C for 4-7 days .The colonies obtained were counted and expressed as colony forming units for plate per hour (CFU/plate /h).

Identification of Actinobacteria

Actinobacteria were isolated from the atmosphere samples and subjected to purification. Adequate phenotypical test set and chemo-taxonomical investigations were used for the identification of the strains (colony and micro-morphological characteristics, pigment production tests, whole cell sugar pattern, cell–wall chemotype, oxidase, catalase, aminopeptidase, benzidine test, OF glucose, lecithinase, lipolysis, proteolysis, hydrolysis of pectin, chitin, hippurate, casein, esculin, gelatine, soluble starch, degradation of xanthine, elastin, arbutin, growth with 2,3,4,5,6,7 and 7.5 % NaCl utilisation of sucrose, m-inositol, D-melibiose, fructose, xylose, galactose, glycerol, ribose, salicin, glucose , arabinose, NO_3- reduction, H_2S production [39 and 12].

Results and Discussion

There is no doubt that the pollution caused by microbial aerosols presents specific hazards to the human occupants. Organic particulate components of the air include plant pollen grains, spores from 20.000 to 40.000 species of fungi, actinomycetes and bacteria [28]. A sedimentation method has used to gain insight into the quantity and quality of actinobacteria in the investigated area. The mean counts (CFU/plate/h) of actinobacteria at the atmosphere surrounding the chamomile plants at El-Fayoum Governorate were shown in Table 1. It increases slightly from the beginning of cultivation period (November) to the 1. of January. Remarkable increase is noticed during the end of January and the beginning of March (harvesting period) followed by a drastic decrease at the late harvesting of the chamomile plants (end of March). These results are in agreement with those of [8 and 11] who reported that crop harvests have been coincided with increased concentration of microorganisms.

A total of 311 actinobacteria colonies from the atmosphere under consideration were obtained and characterised. The distribution of the isolates into different actinobacteria morphotypes is shown in Table 2. The isolated strains fell into 7 genera and 13 species.

Table 1. *Mean monthly count in CFU of actinobacteria from the atmosphere surrounding chamomile plants*

Month	Mean actinobacteria counts CFU/Plate/h
November	93
1st January	103
30th January	213
5th March	314
25th March	129

The dominant genus was identified as Streptomyces (229 isolates and it represents 73.63%). The dominant group with 143 (45.98%) strains could be identified as Streptomyces anulatus. Members of this group have rectiflexibiles or occasionally spiral spore chains, the spore surface is smooth. Melanoid pigments are generally not produced, but a few strains are positive, particularly on tyrosine agar. The members of this group belong to the yellow and grey colour series. Based on a broad spectrum of phenotypical characters this species has been delineated at 81% SSM by Williams and Coworkers [39]. 52 strains (16.72%) are members of Streptomyces albus. This group have spiral spore chains, the spore is smooth and it belong to white series. Melanoid pigments are not produced. 23 strains (7.39%) identified as Streptomyces californicus and 11 strains (3.54%) were identified only at the genus level as Streptomyces sp. Streptomyces californicus have spore chain rectiflexibiles and the surface is smooth. The spore mass is red, yellow to grey. Melanin pigment is not produced. This variable species is widespread in nature.

Table 2. *Total count of genera and species of actinobacteria isolated from the atmosphere surrounding chamomile plants and the percentage of its occurrance*

Genera and species	Number of isolates	% of occurred species
Streptomyces anulatus	143	45.98
Streptomyces albus	52	16.72
Streptomyces californicus	23	7.39
Streptomyces sp.	11	3.54
Micrococcus roseus	27	8.68
Micrococcus luteus	17	5.47
Micrococcus sp.	6	1.93
Rhodococcus sp.	10	3.21
Arthrobacter globiformis	4	1.29
Arthrobacter sp.	4	1.29
Microbacterium sp.	5	1.61
Cellulomonas sp.	5	1.61
Nocardia sp.	4	1.29
Total isolates count	311	100.00

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The specific components or metabolites in the spores of streptomycetes should be considered the most probable causative agents of allergic and/or immunostoxic respiratory disorders. For example, streptomycetes have complex lipid–sugar structures in their cell walls [2]. These bacteria also produce a vast variety of bioactive compounds as secondary metabolites [1]. Streptomyces anulatus and Streptomyces albus are actinomycetes common in soils and dust which may cause allergic alveolities [10]. In addition, the spores of Streptomyces californicus were also found cytotoxic to mouse macrophages [1].

The percentage of the total non streptomycetes isolates was 26.37%. 40 strains of non streptomycete actinomycetes were belonging to the genus Micrococcus which represents 16%. Micrococcus is Gram positive, non sporing, colonies usually pigmented in shades of yellow or red colours and catalase positive. They occur in soils, but commonly they are isolated from air. The first cluster comprises Micrococcus roseus. The second one identified as Micrococcus luteus and six strains could be identified as Micrococcus sp. Several species from Micrococcus are known to have allergic properties [13]. The growth conditions present in the study area regulate its secondary metabolism such as toxin production.

10 strains could be characterised as Rhodococcus (3.21%). Rhodococcus have rods to branched mycelium and Gram positive. They are widely distributed, but particularly abundant in soils.

8 strains are members of genus Arthrobacter (2.58%). Arthrobacter cells are irregular rods, often V-shaped but there are no filaments, Gram positive and catalase positive. It represents marked rod-coccus growth cycle. This genus is widely distributed in the environment, principally in soils. Four of them are Arthrobacter globiformis. Much less is known about the potentially pathogenic properties of corynebacteria associated with organic dust. Nevertheless, cases of allergic alveolitis caused by Arthrobacter globiformis [23] and the involvement of peptidoglycan produced by these bacteria in causing allergic disease can not be excluded.

With the help of our determinative key, Microbacterium sp. (1.61%), Cellulomones sp. (1.61%) and Nocardia sp. (1.29%) could be identified.

Microbacterium have irregular rods, no marked rod-coccus cycle and Gram positive. It is commonly found in soils.

Cellulomonas also have irregular rods, no mycelium is found, Gram positive, catalase positive and it showed cellulolytic activity. It seems to be common in sugar based biopolymer rich habitats, weather on surface of leaves, weather in soil and decaying vetable matter.

Nocardia have rod-shaped to coccoid elements and Gram positive. They are widely distributed and abundant in soils.

The bacteria triggered the production of pro-inflammatory mediators at lower concentration indicating that inflammation may be the primary response in lungs. These results imply that bacterial species need to be considered as causative agents for adverse inflammatory effects in this environment. We can conclude that during harvesting of chamomile plants, microorganisms are released onto the air and high concentration of airborne bacteria may occur inside the harvesting area.

In conclusion, the obtained results indicate that farmers exposed to large concentrations of grain dust and associated microorganisms during harvesting period are under increased risk of work – related pulmonary disorders, such as allergic alveolitis, asthmas, chronic bronchitis and orgasnic dust toxic syndrome.

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