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Study of Microbial Diversity of Three Common Tropical Scented Flowers Rose, Jasmine & Marigold

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Microbial diversity constitutes the most extraordinary reservoir of life in the biosphere that we have only just begun to explore and understand. In this research work, microbial diversity of three tropical scented flowers Rose, Jasmine and Marigold were studied. Representative samples of flowers were collected aseptically from various sites of Bhubaneswar of district Khurda, Orissa during the period from the mid of February to July 2007. Maximum possible number of culturable bacteria, fungi and yeasts were obtained from three stages of flowers and a gradual pattern in the density and diversity of microbes was observed. A total of 71 bacterial strains were obtained from all the three flowers and were categorized into 17 different genera as per their biochemical characterization. Grams reaction of these isolates indicates 42 were Gram -ve rods and 29 were Gram +ve, of which 19 were bacilli and 10 cocci respectively. The predominant bacteria isolated includes *Bacillus sphaericus, Bacillus cereus* in Jasmine, *Bacillus lentimorbus, Serratia plymuthica* in marigold and species of *Flavobacterium, Xanthomonas* in Rose flowers. *Acetobacter aceti* and *E.coli* were isolated from all the flowers. Pigmented bacteria like *Serratia plymuthica* and *Serratia marsescens* have been isolated from the marigold during the course of study. *Curvullaria* sp and *Candida* sp. was isolated from Rose, however only a *Penicillium* sp was isolated from Marigold whereas no fungi or yeast was isolated from Jasmine. In this study the presence of microflora has been elucidated but their significance and the impact on the respective flowers is yet to be revealed.

Key words: Culturable bacteria, Saccharolytic, Pigmented microorganisms.

Flowers are the pleasant creation of this nature with colour and fragnance. About 2.4 lakhs known species of flowering plants are distributed in the various corner of the world. It is one of the ecological niches of diversified groups of microorganisms(Paul *et al.,*1976). The study of microbial diversity of flowers is just at its stage of infancy, which is still a great curiosity for microbiologists. In the modern scenario, microorganisms are widely used for production of different types of colour and scents, for the rising of human need. The curiosity of the relation between flowers and its microflora is accelerated by the discovery of plants such as *Rafflesia*, the

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titan arm and the North America paw paw *Asiminia triloba* which produces a scent imitating rotten meat due the presence of bacteria *Clostiridium botulinum* (Armstrong *et al.,* 1996). On the other side *Puccinia rusti* a fungi has been isolated from *Cestrum nocturnum* (Kevan *et al.,* 1998) which exactly mimics the scent of the flower. Keeping in view of the above facts the present investigation was under taken in order to study the microflora of the three common scented tropical flowers including Marigold, Rose and Jasmine respectively.

MATERIAL AND METHODS

Collection of Sample

Three representative flowers samples including Rose, Marigold and Jasmine were collected aseptically in three different viz.,bud, semiopened and opened stages of flowering from various sites of Bhubaneswar of district Khurda, Orissa during the period from the mid of February to July 2007 for the investigation. The samples were processed aseptically immediately in the laboratory to isolate inhabiting microflora, study their morphology and other characteristics features required for their identification.

Bacteriological analysis of flower

Two different sets of sample were inoculated aseptically in the liquid medium i.e. Nutrient broth at room temperature and 37ºC/24 hours, respectively. Hundred microlitres of inoculums was taken and spread plated on two different media viz., Nutrient Agar (NA) and Acetobacter media for isolation of culturable bacteria and *Acetobacter sp.* respectively. From these bacterial isolates, colonies showing different morphology were selected, subcultured once or twice on Nutrient agar plates to obtain pure culture and were preserved in respective Nutrient agar slant at low temperature (4ºC) for further characterization.

Identification of the bacterial isolates

The bacterial isolates of the three different flowers were identified by studying colony characteristics on different selective media, Gram reaction and identification through a series of biochemical tests and also other features required for their characterization following standard microbiological techniques by (Collins and Lyne, 1970) with further study on their sugar utilization, halophillic nature, enzymatic activities and growth at variant temperature range.

Effect of temperatures on growth of bacterial isolates

The growth of isolates was tested on Nutrient broth by incubating them at a difference of 5°C ranging from 30°C to 70°C. Then they were streaked on NA plates and their temperature resistance was observed from their viability after incubating them at 37°C/24 hours, respectively

Halophilic nature of bacterial isolates

The halophilic nature of bacterial isolates was studied by incubating 100µl of culture in different tubes containing 5ml of NB with varying amount of NaCl at a difference of 1% concentration ranging from 1 to 9 % of sodium chloride (NaCl). After incubating 18 to 24 hours at 37°C, a loopful of the culture was sub-cultured onto NA plates. All the plates along with a control NA plate without NaCl were incubated at 37°C for 18 to 24 hours. The highest concentration of NaCl on which growth occurred, was considered as the maximum halophilic nature of the isolates.

Enzymatic activities of the bacterial isolates

All the isolates were screened on pseudo-selective media for production of various industrially important extracellular enzymes like amylase, cellulase, pectinase, gelatinase, caesinase, lipase, chitinase and DNAase following standard microbiological methods of Collins and Lyne (1970).

Fungal analysis of flower

The fungus and yeast were isolated on PDA and RBA plates respectively, and then identified by lactophenol cottonblue staining.

RESULTS AND DISCUSSION

Total aerobic microbial load of the samples

The bacterial load of the three different flowers of different stages ranged from 1.6×10^1 to 3.2×10^3 CFU/ml. A gradual pattern was noticed in the diversity and number of microbes from the budding to the opened stage of flowers giving an indication that open flower being prone to the variation of external environments and is the shelter house of more microbes as compared to the buds which is enclosed.

Identification and distribution of Microbial isolates

From all the three flowers a total number of 71 bacterial strains were obtained. These 71 bacterial strains ware categorized into 17 different genera as per their biochemical characterization. Grams reaction of these isolates indicates 42 were Gram -ve rods and 29 were Gram +ve, of which 19 were bacilli and 10 cocci respectively.

The individual results of Grams reaction of the three flowers is represented in Fig. 1 (a,b,c).

The predominant bacterial isolates are *Bacillus sphaericus, Bacillus cereus*, *Moraxella saccharolyticum* and *Flavobacterium spiritovorum* in Jasmine. *Achromobacter* sp., *Xanthomonas* sp. and *Streptococci* sp. in Rose. *Flavobacterium* sp. *Serratia marcescens, Pseudomonas aeruginosa, Bacillus lentimorbus*, and *Bacillus cereus* in Marigold flower. *Flavobacterium IIb, Acinetobacter anitratus* and *Rhizopus nigricans*, part of the normal microbiota associated with the marigold flower was reported by Jose *et al*., (2004). However, *Acetobacter aceti* was isolated from all the three flowers which is a common inhabitant found in flowers and fruits (Puspita *et al*., 2001).The number and diversity of microbes accelerated in the semi-opened and opened stages of flowers. Similar results were noticed by Paul in (1976) while studying buds of 10 species of deciduous hard wood trees from which he isolated several species of bacteria and fungi. During the period of investigation pigmented bacteria like *Serratia plymuthica* and *Serratia marcescens* have been isolated from the Marigold flower which were capable of producing red colour pigment on the basal media at low temperature. *E.coli* is isolated from all the three flowers in opened stage whose source may be the

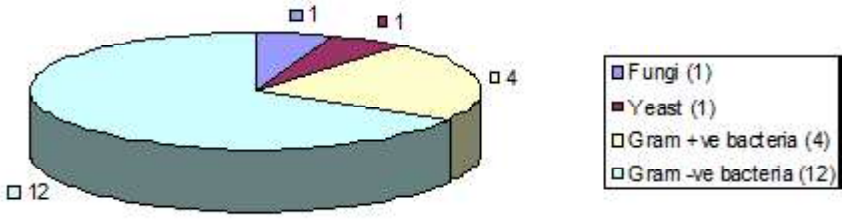

Fig. 1a. Total no. of isolates from Rose Flowers

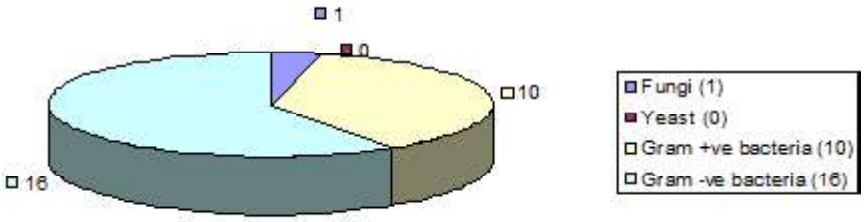

Fig. 1b. Total no. of isolates from Marigold Flowers

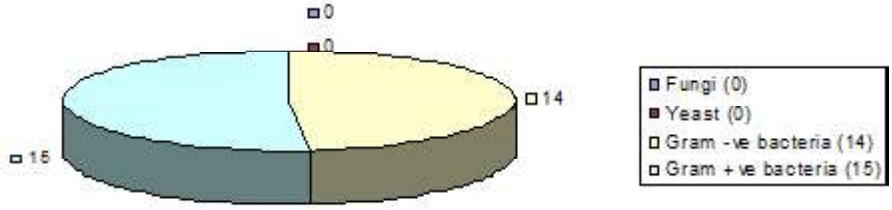

Fig. 1c. Total no. of isolates from Jasmine Flowers

Fig. 1. Total No. of bacterial isolates from the flowers under study

water used in plant growth,a parallel finding was reported by Guo in 2001 in which *Salmonella* sp. present in water contaminate the tomato fruits via flower.

Sugar utilization of the bacterial isolates

Sugar is the most important constituent of various flowers, So microorganisms utilize different sugars in order to meet their energy requirements. Hence, the sugar utilization of the bacterial isolates was studied and it was found that majority of the isolates were positive for Dextrose, Fructose, Sucrose while only 20% and 60% were positive for Dulcitol and Inositol respectively. The detailed result is presented in Fig. 2.

Halophilic nature of bacterial isolates

Regarding the halophillic nature, almost all the isolates have the ability to tolerate 0-3% NaCl concentration and only 26 bacterial isolates survived up to 7% NaCl concentration, where as the number decreased to 3 at 9% NaCl concentration. The detailed results of the halophilic nature of the bacterial isolates are presented in Fig. 3.

Effect of temperature on bacterial isolates

The bacterial isolates of flowers could withstand a wide variation in temperature change. . The optimum growth was observed at 35°C-40°C but bacilli could grow at a temperature of 55ºC, however none of them were able to survive above 65°C which is represented in Fig. 4.

Enzymatic activity of the bacterial isolates

Enzymatic activity of 71 bacterial strains isolated from the three different flowers was observed. The detailed enzymatic activities of all the bacterial isolates are given in Fig. 5. Analysis of the data on the enzymatic activity of all the bacterial strains showed that, upto 70% of the bacterial isolates were positive for amylase, caesinase and cellulase. The enzymatic activity was reduced for lipase, DNAase and chitinase and only 25% are able to produce the enzyme pectinase. Most of bacterial isolates of different flowers showing amylase activity because of high sugar content which acts as sole source of carbon and energy. Bolanos *et al*., (2005) found that

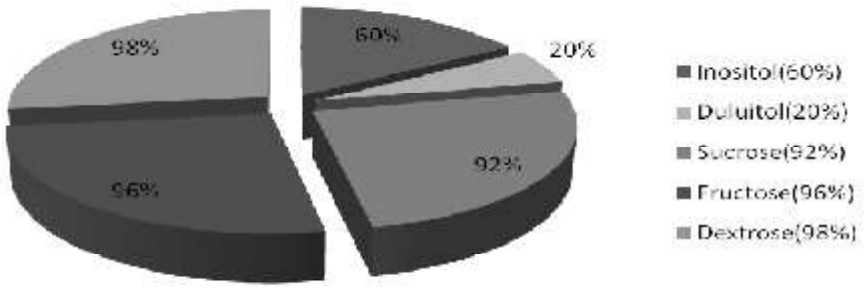

Fig. 2. Percentage of bacterial isolates showing different sugar utilisation

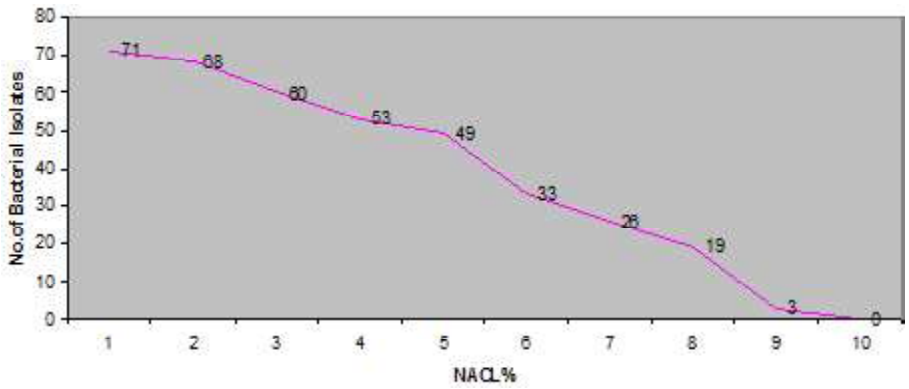

Fig. 3. Halophilic nature of bacterial isolates

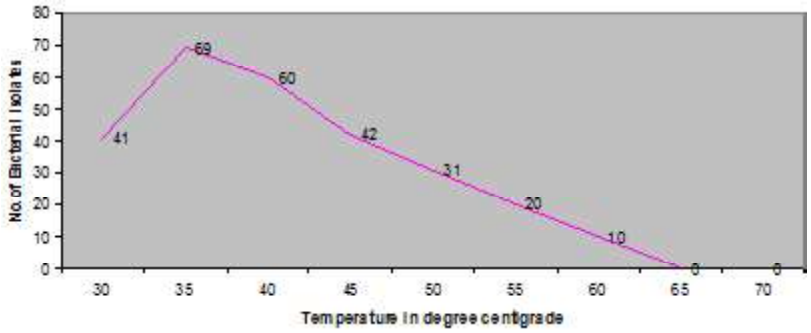

Fig. 4. Temperature resistance of bacterial isolates

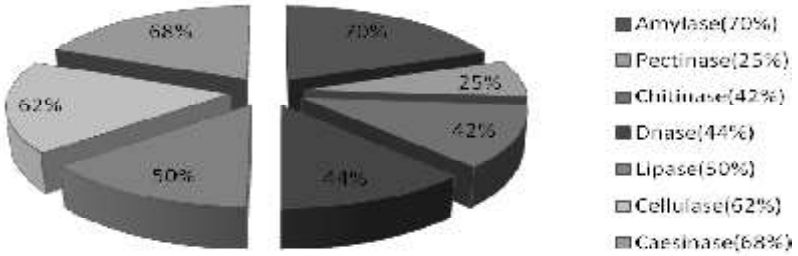

Fig. 5. Percentage of bacterial isolates showing different enzyme activity

certain microbes in marigold have high cellulase activity which is mainly responsible for flower ensilage.

Fungal analysis

Penicillium sp. was isolated from Marigold whereas *Curvullaria* sp. and a yeast belonging to the genera Candida were isolated from Rose They were identified on the basis of conidia and spores by lactophenol cottonblue staining and growth pattern was observed in Candida Hichrome Agar respectively. Corresponding to this finding *Aspergillus* sp. was found in Dhataki flowers Maheshwari *et al.,* (1999) and an anormophic yeast sp. has been isolated from flowers of Rajasthan by Prasad *et al.,* (2007).

CONCLUSION

The most interesting fact is that the flowers are also a shelter of diversified microbial population including bacteria, fungi and yeast. There is a rise in this population from budding to open stage of the flowers. Most isolates are potential sugar degraders fermentative and non-fermentative, temperature resistant and enzymatically active .The isolation of pigmented microbes gives a cue to analyze their role at the molecular level. Further study of their interaction may pave a pathway for any probable commensal or mutual linkage in scent and pigment production of microbes and flowers..The concept can be applied industrially for production of colour and fragrance. Further proceedings will elucidate the significance of this microflora in flowers in a more understanding manner.

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