International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.11, pp 128-132, 2016

Study of Microbial Diversity of Rosa Centifolia

Nisha Kumari, Biji A. Varghese, T. Rashmi, Pramita Ghosh, Abhishek Suman, Anjuvan Singh*

Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara 144411, Punjab, India

Abstract : Increasing loss of floral fragrance over a long duration has provided an opportunity to study the microbiological aspect of the flowers. Besides having ornamental value, roses are also used commercially to distil the fragrance for the production of numerous value added products. This study is an attempt to identify microbiota of *Rosa centifolia* that might be responsible for the loss of their natural fragrance. A total number of five bacterial strains comprising of Gram negative, Gram positive and acid fast bacilli, were isolated followed by biochemical characterization for identification of bacterial genus. The tests for characterization involved endospore staining, catalase test, motility test, starch hydrolysis test and glucose fermentation test. The identified dominant bacteria included *Pseudomonas* spp., *Escherichia* spp., *Streptococcus* spp., *Bacillus* spp.,and*Shigella*spp., out of which *Pseudomonas* isa scented bacterium. *Bacillus* and *Escherichia* were the most profuse isolates the isolated microbial population. Study for halo-tolerance revealed that all isolates had moderate tolerance for NaCl. A further study to determine the biochemical effect of microbial interaction with fragrant volatile molecules of flower is in progress.
Keywords : Rose, Microbial population, Isolation, Scented microbes.

Introduction and Experimental

Scented species of genus *Rosa* are of ornamental and commercial importance as the distilled fragrance of their petals and nectar is used for production of highly-prized essential oils, perfumes and other cosmetic products, apart from gardening**.** Rose is said to be as the most important flower for its appearance and fragrance in industry [1]**.** Among scented roses, there were four species, namely *R. damascene* M., *R. gallica* L., *R. centifolia* L., and *R. moschata* H. The studies on the aroma of flowers have mainly focused on its biochemical illustrations describing the role of various biosynthetic pathways in the production of a complex of intermediate low-molecular-mass volatile molecules responsible for distinctive fragrance. [2,3] There are numerous plant species which have very important role in day-to-day life of ethnic and local people[4].

However, it is laborious to predict the fragrance of flower, but over a long duration, a gradual decrease in natural aroma of flowers has been observed [5]. Among breeders and retailers, it is a matter of high concern due to which it has drawn the attention of many researchers. Despite the numerous benefits, probably, the concept of crossbreeding has also led to the unintentional development of various plants with compromised durability and fragrance, due to some unfavourable interactions between these characteristics but the specific causes of fragrance loss are still unknown. Two scented parents may produce an unscented or inadequately scented progeny, based on dominancy of responsible alleles [6,7] .

Being a rich source of carbohydrates, flowers is one of the ecological habitats of diversified microbiota [8]. The relation between flowers and microbes has taken a new turn by the isolation of *Puccinia* rust fungus from *Cestrum noctumum*, which exactly mimics the fragrance of the flower. Some micro-organisms, e.g. *E. coli* BL21(D3), have also been engineered to produce fragrance imitating that of roses when induced by isopropyl thio-galactosidase [9,10]. Recently, attention has been given to utilize biodegradable and bio-friendly based plant product for the prevention and treatment of various human diseases [11]. The synthesis of silver and nanoparticles has been done by biomimetics pathway using rose leaf, natural rubber, starch, mushroom, alfalfa leaf and other bacterial, viral materials [12]. The present work elucidates the microbiota of petals of *Rosa centifolia* (Family: Rosaceae; a hybrid bisexual short shrub flowering annually).

Experimental

Collection of plant materials and sampling:

Two days old completely flourished flowers of *R. centifolia*were plucked from the botanical garden of the Lovely Professional University, Punjab campus (Figure 1). Under aseptic condition, 10g of dissected petals were suspended in 10ml of sterilized normal saline (0.85% NaCl; pH 7.0) solution for 1 hour at room temperature. This solution was used for isolating bacteria.

Figure 1: Petals of *R. centifolia* used in the experimentation.

Medium preparation and inoculation:

The medium was prepared by dissolving 2.8g of Nutrient agar (HiMedia) powder in 100ml of distilled water, followed by sterilization by autoclaving at 15lbpsi (121°C) for 15 minutes. Maintaining sterility, 20ml of the medium was uniformly distributed in 3 petri-plates. After solidifying and cooling, each nutrient agar plates were inoculated by spreading 100µl of the above mentioned sample and incubated at 37°C for next 48 hours. The colonies appeared on the plates were streaked on different plates of nutrient agar medium and incubated at 37°C for next 48 hours. Form different plates, colonies were dissolved separately in 5ml of sterile normal saline.

Tests for biochemical characterization of micro-organisms:

The above mentioned bacterial suspensions were used for biochemical characterization of the micro-organisms. The tests for characterization involved Gram staining, endospore staining, acid-fast staining, catalase test, motility tests, starch hydrolysis test (Figure 2 and carbohydrate fermentation test (using glucose, sucrose, lactose and mannitol; only for Gram negative isolates; table 1). The last test was also used to determine the ability of isolates to utilize different carbohydrates for growth.

Table 1. Morphological, staining and biochemical characterization for identification of bacterial genus

Tests	**Results**				
Staining and microscopy					
Morphology	Rod	Rod	Sphere	Rod	Rod
Gram stain	-	-	+	+	-
Endospore stain	-	-	-	+	-
Acid fast stain	-	-	-	+	-
Biochemical characterization					
Catalase	+	+	-	+	+
Motility	+	+	-	+	-
Starch hydrolysis	-	-	+	+	+
Glucose fermentation					
Glucose/ (Gas)	- (-)	+ (+)	*	*	+ (-)
Sucrose	-	-	*	*	-
Lactose	-	+	*	*	-
Mannitol	+	+	*	*	+
Bacteria Identified	*Pseudomonas* spp.	*Escherichia* spp.	*Streptococcus* spp.	*Bacillus* spp.	*Shigella*spp.

(+ = Positive; - = Negative; * = Not done)

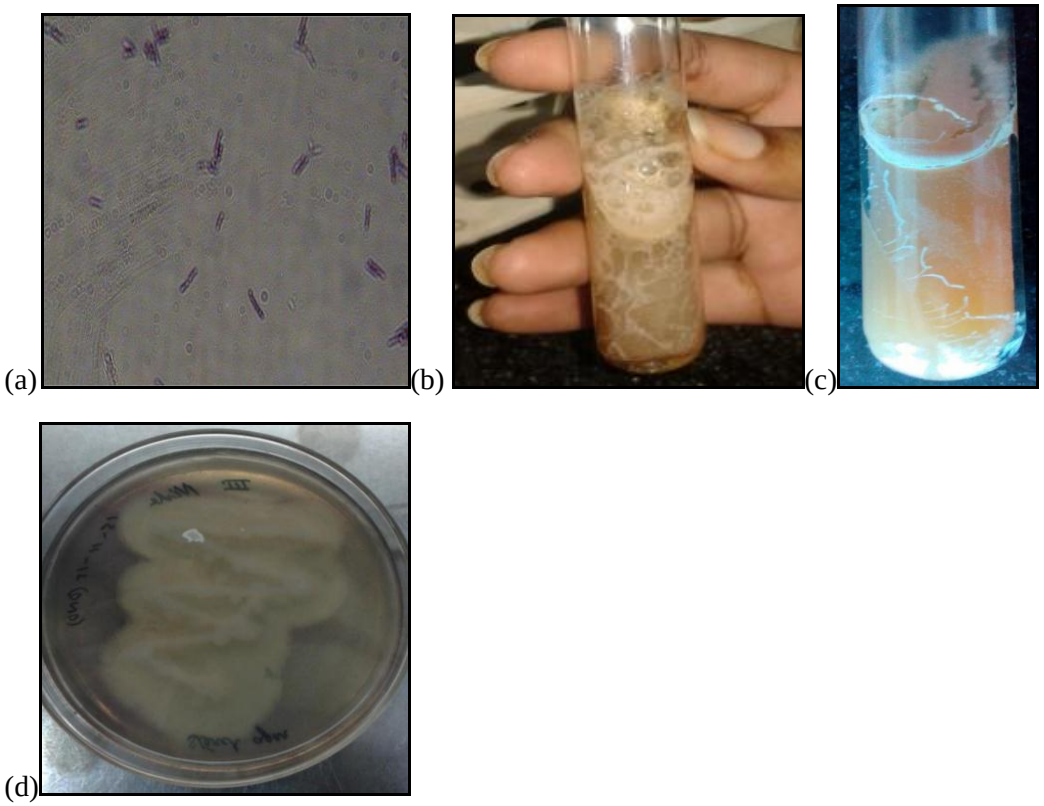

Fig. 2. Observations of positive results for gram staining and biochemical tests: (a) Gram positive *Streptococcus*, (b) Catalase test, (c) Motility test and (d) Starch hydrolysis test.

Halophilic nature of bacterial isolates:

In 5ml of nutrient broth (pH 7.0) with NaCl (1 to 9%; w/v), 50µl of bacterial suspension was inoculated and incubated at 37°C for 24 hours. After incubation, a loop of the culture was streaked onto nutrient agar (pH 7.0) plates. The highest concentration of NaCl sustaining bacterial viability, was considered as the maximum tolerance concentration of the isolates.

Results and Discussion

Total load of aerobic microbes in sample:

A total microbial load of 400-600 cfu/ml was obtained from the petals of *R. centifolia*, which included bacteria of 4 families. Our study showed that five distinct aerobic bacteria, including one Gram positive, three Gram negative and one acid fast bacilli, inhabit on the petals surface of *R. centifolia*. On the basis of morphological studies and biochemical characterization the Gram positive isolate was identified as *Streptococcus* spp [13]. The acid-fast positive isolate was identified as *Bacillus* spp. It is difficult to distinguish between Gram negative isolates, as they exhibit similar biochemical characteristics, except *Pseudomonas* is glucose and lactose non-fermenter whereas *Escherichia* ferments glucose and produce acid and gas. *Shigella* is also a glucose fermenter, but doesn't produce gas. Fermentation was confirmed by change in colour of phenol red from red to yellow and gas production was observed by entrapment of gas bubble in the Durham tube placed inside the phenol red medium. All the three Gram negative isolates didn't ferment sucrose. *Shigella* also showed positive result for starch hydrolysis test, whereas other two isolates showed negative.

Halo-tolerance of the isolates:

Most natural environmenthas high concentration of salts, particularly sodium chloride, which exhibits high osmotic pressure leading to cell shrinking and death. All the isolates, when incubated for 18 hours at different concentrations of NaCl, showed tolerance up to 9% NaCl (Figure 2), which shows moderate tolerance of the microbes for NaCl.

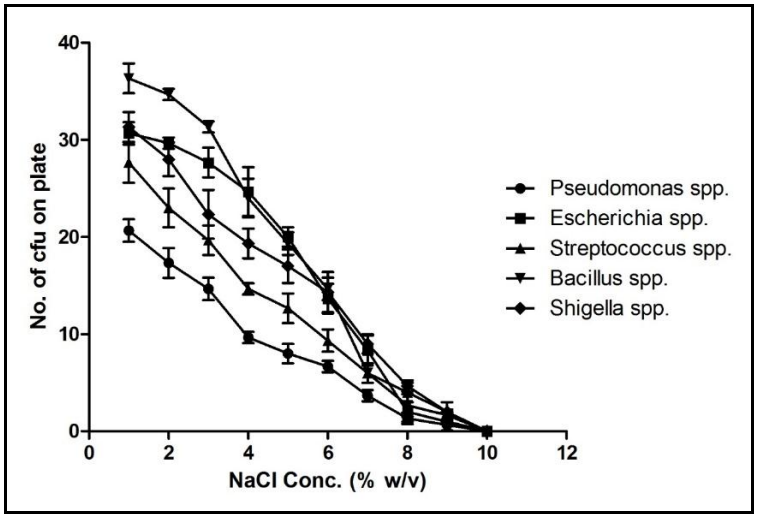

Fig. 3. Halo-tolerance of different microbes isolated from petal surface of *R. centifolia.*

Although roses have bactericidal properties, it also serves as a natural habitat of diverse microbes, some naturally having fragrance properties[14]. The main objective of the research was to analyse the microbiota of the rose petals. The presence of micro-organisms of different genus and having different biochemical characteristics, along with *Bacillus* and *Escherichia* as the most profuse isolates of microbial populations, signifies that numerous microbes inhabit on the rose petals. It has been reported that the extracts of *Rosa centifolia*, because of its antibacterial property can be used for cleaning and sterilization purpose in hospitals and toilets[15].

It is possible that these micro-organisms uptake and metabolize the fragrant volatile molecules produced by the rose petals, leading to the loss the floral fragrance. The suppression of floral aroma by the microbial fragrance might be another possible reason for explaining the fragrance loss. However, these speculations need further study before getting on any final conclusion, but the possibilities can't be denied. If so, the growth of such microbes on the flowers can be prevented by various available approaches, to preserve the natural fragrance of the flowers. In our study, we had also isolated one scented micro-organism, i.e. *Pseudomonas* spp[16]. This gives a clue to consider a different approach in which the micro-organisms that inhabit on the petals of *R. centifolia* can be genetically transformed into strains imitating rose fragrance. Both approaches can be beneficial in increasing the floral aroma of the rose, however, in the later approach, the pathogenicity of the bacterium must be taken in consideration. A further study to determine the biochemical effect of microbial interaction with fragrant volatile molecules of flower is in progress.

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