Yeasts Associated with Pollinating Bees and Flower Nectar

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Abstract. A study of the yeast flora of 328 honey stomachs from 7 different pollinating bee species, and 342 flower nectar samples of 9 different flower species yielded 766 yeast isolates composed of 16 genera and 47 species. Most of the yeast species from both the sources belonged to the genus *Candida*, while the most frequently isolated yeasts were *Dekkera intermedia* from honey stomach and *Candida blanca* from flower nectar. Among the honey bees, *Xylocopa* sp., and among flowers, *Citrus medica*, yielded the highest number of yeast species. Nineteen species of yeasts belonging to 9 genera were common to both the sources.

Introduction

The occurrence of yeasts in nature is widespread. They have been isolated from soil, air, plant and animal surfaces, preserved foods, and sugary substrates, including flower nectar and fruit juices [10, 12, 16]. The association of yeasts with insect guts is well known [3], and pollinating bees in particular harbor yeasts in their nectar sacs. Similarly, flower nectar constitutes a rich source of yeasts. The association of particular yeast species with flowers and insects is known [2, 6, 16] because a number of insects bring about pollination and simultaneously transfer yeasts to flower nectar during their visits to entomophilous flowers. The present study has been aimed at a survey of the yeasts associated with honey stomachs of some pollinating bees and nectary glands of flowers of cultivated or wild plants.

Materials and Methods

Collection of Samples

Seven different pollinating bees, i.e., *Apis dorsata, A. mellifera, A. florae, A. indica, A. cerana, Xylocopa* sp., and *Halictus* sp., were trapped outdoors in the fields from insect-pollinated, cultivated, or wild plants. The bees were kept in the refrigerator after collection to make them inactive and easy to handle. The nectary glands of 9 different flowers, i.e., *Citrus medica, C. acida, Murraya exotica, Raphanus sativus, Brassica juncea, B. rapa, B. campestris, Eruca sativa*, and *Euphorbia helioscopia*, were investigated for the presence of yeasts. The flowers were collected from the fields of suburban Amritsar in sterile polyethylene bags, brought to the laboratory, and processed immediately.
Processing of Samples

Pollinating bees and flowers were dissected aseptically to remove the nectar sacs and nectary glands, respectively, which were transferred to 5 ml of sterile distilled water and shaken to obtain a uniform suspension. The appropriate amount of inoculum was streaked liberally on yeast-extract-malt-extract agar [20] plates which were incubated at 28°C for 72 hours. The colony types appearing on the culture plates were isolated on yeast-extract-malt-extract agar slants on the basis of gross colonial and microscopic morphology of vegetative cells. The identification of the yeast isolates was done according to the methods of Lodder [13] and Barnett and Pankhurst [1].

Results

The physiological characteristics of the yeasts isolated from honeybees and flower nectar were more or less similar, but with some differences. About 10% more fermentative yeasts were isolated from flower nectar. All 13 substrates used in the identification procedure [13] were fermented, with the exception of soluble starch. Glucose and sucrose were fermented by the majority of the yeasts. None of the yeasts from bees fermented melibiose, while only 1 from the flower nectar had this ability.

Prevalence of Yeasts in the Honey Stomach of Pollinating Bees

A total of 328 samples of honey stomach of pollinating bees was investigated. All the samples were culturally positive, yielding 473 isolates, belonging to 13 genera and 30 species (Table 1). The two most common species encountered were Dekkera intermedia (6.71% of the samples positive) and Saccharomyces cerevisiae (6.1%). Among the other common species were Candida humicola, C. incommunis, C. ishiwadae, and C. membranaefaciens, each occurring in 5.5% of the samples investigated. The prevalence of other species varied between 1.57 and 4.57%. One-third of the species identified belonged to the genus Candida, while Cryptococcus was represented by 4 species, Rhodotorula by 3, Debaryomyces, Hansenula, and Torulopsis by 2 species each, and the remaining 7 genera by 1 species each. The highest number of yeast species was isolated from Xylocopa sp. (13 spp.), followed by A. mellifera (11 spp.), A. dorsata (9 spp.), A. florae, A. indica, and A. cerana (6 spp. each), and Halictus sp. (5 spp.).

Prevalence of Yeasts in the Nectary Glands of Flowers

Out of the 342 samples of nectary glands of flowers, 232 (67.84%) were culturally positive, yielding 293 isolates (Table 2). These belonged to 12 genera and 36 species. The most frequently isolated species was C. blankii, occurring in 4.68% of the samples, followed by C. cacaoi and S. cerevisiae, each isolated from 4.09% of the total samples investigated. The genus Candida was found most often, with as many as 16 species, while Hansenula was represented by 4 species, Debaryomyces and Rhodotorula by 3 species each, Pichia and Torulopsis by 2 species each, and the remaining 6 genera by one species each. The nectary