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The elucidation and bioengineering of the vanillin biosynthesis in the vanilla orchid

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Vanilla and its key flavor component vanillin, is a universally appreciated flavor, a global delicacy and probably the most popular plant natural product, being derived from the seedpods of the orchid *Vanilla planifolia* and other related *Vanilla* species. The flavor and fragrance profile of the vanilla extract contains more than 200 components. Vanillin (3-methoxy-4-hydroxybenzaldehyde) is the main flavor compound in the vanilla extract and is the basis and an additive of sweets, ice creams, soft drinks and many more products in the food, beverage and pharmaceutical industry. Despite its popularity, the biosynthetic pathway of vanillin has remained elusive until now. Our studies during last four years reveal how the vanilla orchid produces the most popular aroma compound in the world. A single hydratase/lyase type enzyme designated as vanillin synthase (VpVAN) catalyzes direct conversion of ferulic acid and its glucoside into vanillin and its glucoside, respectively. The enzyme shows high sequence similarity to cysteine proteinases and is strictly specific to the substitution pattern at the aromatic ring. Transient expression of VpVAN in tobacco and stable expression in barley in combination with the action of endogenous alcohol dehydrogenases and UGTs result in vanillyl alcohol glucoside formation from endogenous ferulic acid.

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Variability of *Bacillus thuringiensis* Mexican strains by ERIC-PCR and biofilm formation

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B acillus thuringiensis (Bt) is of important agronomical research interest because of its beneficial use as biological pesticide. There are some limitations regarding the subspecies classification. Studies at phenotypic and genotypic levels are important to ascertain its variability. The aim of this study was to evaluate the variability by ERIC-PCR and by biofilms formation among strains from Mexico. The relationships between 40 environmental strains from the collections of the Cinvestav-Irapuato and IBT-UNAM were evaluated by ERIC-PCR and the biofilm-forming ability by a 96-well microplate-based assay at 72 and 96 h of incubation. 39 fingerprinting patterns, based on 24 polymorphic fragments (139 to 2468 bp) were generated and used to construct a dendrogram. Almost all strains (95%) formed biofilms after 96 h of incubation, whose OD₆₂₀ data were stratified into four categories as follows: 32.5% of them were strong (OD₆₂₀>1.03), 35% were moderate (OD₆₂₀ 1.03-0.52), 27.5% were weak (OD₆₂₀ 0.51-0.27) and 5% were null (OD₆₂₀ \leq 0.26). The subset of strains from the Cinvestav collection showed more heterogeneous biofilm-forming ability. A large intra-species genomic variability was observed among Bt isolates. At 96 h of incubation, most strains from the Cinvestav collection showed moderate to strong biofilm forming ability, whereas those from IBT-UNAM collection were mainly weak biofilm producers. Results showed a large intra-species genomic variability in Bt. However, some strains could be correlated as they were found within clusters depending on the location of isolation.

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