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EFFECT OF RHIZOSPHERE ON PRODUCTIVITY OF VITIS VINIFERA.

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ABSTRACT

The root system of higher plants is associated not only with an intimate environment composed of organic and inorganic substances but also with a vast community of metabolically active microorganisms. This paper investigates the enzyme characterization of bacteria from rhizosphere of Vitis vinifera. For the present work plant with high berry production was selected. Soil sample was collected aseptically from the rhizosphere, rhizoplane and non-rhizosphere, and different dilutions were made. Different selective media like Pseudomonas isolation agar, Minimal agar, Actinomycete isolation agar, Nutrient agar and Saborauds agar were used for isolation of rhizospheric microorganisms. Total 104 isolates of fungi and bacteria were isolated from rhizospheric soil and only 39 isolates were obtained from non- rhizosphere soil. These rhizospheric and non-rhizospheric isolates were characterized for enzyme production by using skim milk, chitin agar, xylan agar, tributyrine agar, starch agar, Pikovskaya medium, Czapek-mineral salt medium, Urea agar. The obtained results indicate that the isolated colony types like NAS I, MMS I, MMS III, MM1I, NAS V, NA1 III shows versatility in enzyme production. These types produce more than one types of enzymes. The obtained results indicate that the increase in berry production is due to the synthetic activity of rhizospheric microorganisms which helps in solubilisation of higher metabolic components to simple form so that they can be easily utilizable and will enhance plant growth and production.

***Keywords*- Rhizosphere, Vitis vinifera, Enzyme characterization, protease**

INTRODUCTION

Rhizosphere is the soil nearest to the plant root system where roots release large quantity of metabolites from living root hairs or fibrous root systems. It is the hot spot for microbial interactions. Bacteria which are able to inhabit this area colonize very efficiently the roots or the rhizosphere soil of crop plants (Vazquez P. et. al., 2000). The released metabolites from these bacteria act as chemical signals for motile bacteria to move to the root surface. They also represent the main nutrient sources available to support growth and persistence in the rhizosphere (Nawani N. et. al., 2003). Different mechanisms are involved in plant growth which is often indirectly connected for the suppression of plant pathogens.

Root exudates from plants stimulate heterotrophic growth, which leads to local competition between roots and microorganisms for inorganic nutrients. Many soil biochemical processes, soil ecology, growth and health of plants is influenced by root-associated microbial community. Physicochemical characteristics of soil, especially soil nitrogen (N) availability and soil pH have a large effect on the composition of soil microbial communities (Eui S. et. al., 2002) (Beom K. et. al., 1999).

Grapes being rich in many biological compounds have many health-promoting benefits for humans such as cardio-protective, antioxidant, anticancer, antimicrobial, anti-inflammation, anti-aging properties. For good market consumption grapes should have a good flavour, an attractive appearance and be rich in functional substances. The quality of grapes can be influenced by many factors, such as cultivation techniques, climatic conditions, soil, aging and region. Different factors decide quality of grapes such as vineyard productivity, berry maturity, rootstock, health, floor, canopy management, and growing environment of the plant (Jong S. et. al., 2003). For understanding of the relationship between rhizosphere soil bacterial community and grape berry quality the present work aims to characterize the isolates for different enzyme production in rhizosphere as well as non-rhizosphere soil. The enzymes like protease, amylase, xylanase, urease, chitinase, cellulase production was tested. The target microorganisms in this study were aerobic because of their potential easier use for mass culture in commercial volumes without the challenges associated with anaerobic bacteria. Vineyard one with high berry production and other with no production (control plant). The collected roots and soil were stored in sterile test tubes and kept at 4^0C until processing.

Materials and Methods-

Collection of samples

The rhizospheric soil sample was collected from the desired plant that is *Vitis vinifera* (Sonaka variety*)* from Vilholi, Maharashtra.

Method

Standard microbiological methods were used to isolate bacteria from rhizosphere and rhizoplane of the plant. The plant roots were cut in smaller pieces of 1-2 cm length and washed twice for 2 minutes in 10 ml of normal saline. The combined washing solutions were centrifuged and the supernatant is treated or referred as rhizosphere fraction (Sharma N. et. al., 2017). Rhizosphere fraction was diluted and plated on selective media like *Pseudomonas* isolation agar, Minimal agar, Actinomycetes isolation agar, Nutrient agar and Saborauds agar. After incubation for 48 hrs colonies were isolated morphologically and all the isolates were further screened for various physiological and biochemical activities (e.g. enzymes production – protease, xylanase, esterase, amylase, cellulase, DNase, phosphatase, oxidase, urease and catalase) profiled for different enzyme production (Beom K. 1999).

Sr No.	Enzyme	Media used for isolation
1	Protease	Skim milk agar
2	Xylanase	Xylan agar
3	Chitinase	chitin agar
4	Esterase	Tributyrine agar
5	Amylase	Starch agar
6	Cellulase	Czapek-mineral salt medium
7	Urease	Urea agar
8	Phosphatase	Pikovskaya medium

Table-1. Different medias used for enzyme characterization.

Results-

After inoculating the isolates on different media, specific for detection of enzyme production following observations were obtained.

Fig-1 Colonies showing clear zone on Skim milk agar

Table 2- Isolates showing production of selected enzymes from rhizospheric soil

Sr No.	Enzyme	Colony type
1	Protease	NAS I, NA XI, NA2III, NA2 V, NA3 IV, MMS I, MNS III, MNS V, MNS VI, MN1, MN III, MM1 VII, MM3 II, A12 I, A12 VII = 15
2	Xylanase	NA1 X, NA2 V, MMSI, A12 I = 4
3	Chitinase	MMI, MMP1 II = 2
4	Esterase	NAS I, NAS V, NA1 X, NA2 V, NA3 I, NA3 III, MMSI, MMS II, MM1 I, MM1 III, MM1 VII, A12 I, A12 VII, A12 I, A12 VII, A 15 II =16
5	Amylase	NA1 III, NA1 XI, NA3 IV, MMS I, MMS III, MMS IX, MM1 I, A12 III, A12 VII
6	Cellulase	NAVII, MM3 I, A11 V =12
7	Urease	NAS I, NASII, NAS III, NAS IV, NAS V, NAS VI, NA1 IX, NA2 I, NA2 II, NA V, NA3 I, NA3 III, SB3 III, SB5 I =14
8	Phosphatase	NA1 III, NA1 IX, NA2 V, MMSIII, MMSVII, A11 V=06

Table 3- Isolates showing production of selected enzymes from non-rhizospheric soil

Sr. No.	Enzyme	Production
1	Protease	AIP2 III, NAPS II=02
2	Xylanase	----------
3	Chitinase	MMP1 II, NAP2 III=02
4	Esterase	NAPS II, NAPS I=02
5	Amylase	----------
6	Phosphatase	NAPS II=01
7	Cellulase	NAP1 I=01
8	Urease	NAP1 I, NAPS II, SBP1 II,PI1 I, AIP3 I=05

Legends: - NA - Nutrient agar, MM - Minimal medium, SB - Saborauds agar, AI - Actinomycetes isolation agar, PI – Pseudomonas isolation agar.

The observations obtained above are suggestive of the selective effect of rhizosphere, which mainly favours growth of different organisms which are more active physiologically than those from corresponding control soil. The rhizosphere region is a highly favourable habitat for the proliferation and metabolism of numerous microbial types. These microbes belong to several distinctly different physiological, taxonomical and morphological groups. The bacterial density in the rhizosphere is enormous. There must seems a rhizospheric soil are more diversified in their activities than those of non-rhizospheric soil. The synthetic activity of bacteria of rhizosphere with respect to enzyme production is beneficial to plant growth and to increase berry production. The rhizosphere contains a bacterial flora which is more active physiologically than that of non rhizospheric soil. Bacterial and fungal diversity increases soil quality by affecting agglomeration and increasing soil fertility. The obtained results indicate that the rhizospheric microorganisms helps in solubilisation of higher metabolic components to simple form so as it can be easily utilized by plants and shows significant growth and production of berries.

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