International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(6): 2868-2873

Received: 14-10-2020
Accepted: 21-11-2020

VR Bangar
Ph.D., Scholar, Department of Plant Pathology and Agril, Microbiology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

Dr. SV Kolase
Associate Professor, Department of Plant Pathology and Agril, Microbiology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

Dr. SB Sable
Ph.D., Scholar, Department of Plant Pathology and Agril, Microbiology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

Dr. SB Latake
Scientist, Department of Plant Pathology, Pulses Improvement Project, MPKV, Rahuri, Maharashtra, India

Corresponding Author:
VR Bangar
Ph.D., Scholar, Department of Plant Pathology and Agril, Microbiology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

Isolation and identification of Triazole group fungicide degrading isolates from grape rhizosphere of major grape growing districts of Maharashtra, India

VR Bangar, SV Kolase, SB Sable and SB Latake

DOI: https://doi.org/10.22271/chemi.2020.v8.i6ao.11342

Abstract
Grape (*Vitis vinifera* L.) is the most important temperate fruit crop that has acclimatized to the subtropical and tropical agro-climatic conditions. Thirty soil samples were collected from grape vineyards rhizosphere of different locations of Nashik, Ahmednagar, Pune, Solapur and Sangli districts of Maharashtra during 2017-18. The 17 isolates (FDB1 to FDB17) were obtained from soil samples which had ability to degrade difenoconazole, myclobutanil and fluopyram+ tebuconazole fungicides. On the basis of colony characteristics as well as biochemical test, eight Gram +ve strains and nine Gram -ve strains were identified up to the generic level. The Gram +ve strains were 3 strains of *Micrococcus* spp., 2 strains of *Bacillus* spp., a strain of *Staphylococcus* spp., *Inquilinus* spp. and *Lysinibacillus* spp. In Gram –ve strains *viz*., eight strains of *Pseudomonas* spp. and a strain of *Stenotrophomonas* spp. were identified which has ability to use sole carbon source from fungicide for its growth.

Keywords: Difenoconazole, myclobutanil and fluopyram+ tebuconazole, *Micrococcus* spp., *Bacillus* spp., *Staphylococcus* spp., *Inquilinus* spp., *Lysinibacillus* spp., *Pseudomonas* spp. and *strenotophomonas* spp

Introduction
Grape is commercially cultivated in the tropical wet and dry or the arid and semiarid climatic regions of peninsular India where the weather is conducive to support insect pest attacks on grapevines during both the vegetative and fruiting seasons. The important grapevine diseases are powdery mildew, downy mildew, anthracnose, dead arm, gray mold or bunch rot, black rot, crown gall and bacterial leaf spot. Various fungicides are commonly used to manage diseases of grapevine.

Powdery mildew, caused by the biotrophic fungus *Erysiphe necator* (Schw.) Burr (earlier *Uncinula necator*), is one of the most important fungal diseases of grapevines. In table grape vineyards located in Maharashtra state, India, powdery mildew infections can be seen almost throughout the year, except during the hot and dry months of summer season and for a brief period during the cold winter months. Thompson Seedless and other commercial table grape varieties grown in this region are highly susceptible to powdery mildew (Sawant and Sawant, 2012) [20].

Frequent pesticide applications are necessary to control the different pests and prevent qualitative and quantitative losses. Powdery mildew management, thus, is very challenging and constraints the grape growers to apply the recommended fungicides at frequent intervals to achieve the desired level of disease control. Difenoconazole, myclobutanil and fluopyram + tebuconazole are relatively broad spectrum systemic fungicides, belonging to the triazole family of chemicals, and are commonly used for the control of powdery mildew in vineyards.

Triazole group fungicides are known to be fairly soluble in water, although they are not readily degradable and have a limited sorption tendency. Triazole fungicides are toxic and persist in the soil for long periods of time, thus affecting soil fertility and microflora (Elmholt, 1992 and Munier and Borde, 2000) [7, 12].

Microorganisms are most desirable biological tools, because of their ability to resist various pesticides and their metabolic capacity to degrade toxic compounds into nontoxic forms.

Hence, soil microorganisms are considered a key reservoir of biological activity with the potential to significantly enhance environmental cleanup (Dong *et al*., 2008; Satapute *et al*., 2012 and Kulkarni and Kaliwal, 2014) [4, 19, 10]. The microbial strains can be isolated and identified to achieve the required result like biodegradation of pesticide in short time frame. In field, multiple microbial consortia via either selective enrichment or chemo stat enrichment can be used for mineralization of specific pesticide at faster rate. The bioremediation has a better future in development of technology for removal of contaminants from actual site (Singh, 2008) [23].

In earlier studies, some scientists reported that the strains of *Bacillus* spp. isolated from grape ecosystem, are able to enhance the *in vitro* degradation of triazole fungicides (Salunkhe *et al*., 2015c) [15]. *Pseudomonas aeruginosa* strain had degrades the propiconazole (Satpute and Kaliwal, 2016a). Like many other pesticides which are metabolized by microorganisms for food or energy (Arbeli and Fuentes, 2010) [2]. Many pesticide degrading microorganisms had been reported belonging to various species of bacteria, fungi, algae and yeast. However, bacterial bio-remediation studies have been more successful because of the diversity of their metabolism and their ability to grow on complex carbon substrates (Satpute and Kaliwal, 2016a). It is reported that a wide range of micro-organisms under different environmental conditions are capable of degrading triazole group of fungicides (Eizuka *et al*., 2003; Sehnem *et al*., 2009 and Sarkar *et al*., 2009) [6, 21, 17] which are systemic in nature but are mainly accumulated in the peel. The residue accumulations on the surface of fruits make them amenable to biodegradation (Angioni, *et al*., 2003) [1]. Therefore, ecofriendly and feasible approaches such as microbial biodegradation are gaining importance.

Considering the economic importance of the crop, environmental fates due to extensive use of fungicides, fungicide contaminated soil and fungicide residues in grapes, present studies was planned and conducted to isolate and identify the fungicide degrading microorganisms from grape rhizosphere.

Materials and Methods

Fungicides

The tested fungicides were obtained from the local market. Particulars of evaluated fungicides are as Difenoconazole 25% EC, Score, Syngenta India Ltd.; Myclobutanil 10% WP, Systhane, Dow Agro Sciences Ltd. and Fluopyram 17.7% + Tebuconazole 17.7 w/w SC, Luna Experience, Bayer Crop Science Ltd.

Collection of soil samples

The 8 to 10 years old grape orchards having a more pesticide sprays history were selected for collection of soil samples from major grape growing areas of Maharashtra state, India. A roving survey for collection of soil samples from grape vineyards were conducted during the year 2017-2018 in five districts of major grape growing areas under M.P.K.V. university jurisdiction *viz*., Nashik, Ahmednagar, Pune, Solapur and Sangli in the months of December to February (peak period of disease). Two - three tehsils per district, one village per tehsil, two to three plots per village location were selected randomly. Soil samples were collected from topsoil of the grape vineyard ranging in depth from 10-15 cm. one composite sample was collected from each surveyed village. The composite samples collected in polythene bag and sample codes were given to each sample and brought to the laboratory and half samples stored at room temperature and remaining kept at 4 ^{0}C in refrigerator until processed.

Isolation, incubation and purification

Thirty soil samples were air dried at room temperature and were allowed to retain 20% (w/w) moisture content. These soil samples were passed through sieve with 2 mm mesh. Mineral salt medium (Seubert, 1960) [22] was prepared by a composition are K_2HPO_4 (6.30 g), KH_2P0_4 (1.82 g), NH_4N0_3 (1.00 g), $MgS0_4.7H_20$ (0.20 g), $CaCl_2.2H_20$ (0.10 g), $FeS0_4.7H_20$ (0.10 g), $Na_2Mo0_4.2H_20$ (0.06 g), $MnS0_4.7H_20$ (0.06 g) and Distilled water (1000 ml). The MSM was boiled, filtered and the pH was adjusted to 7.0. The medium was then dispensed in 100 ml quantities in 250 ml Erlenmeyer flasks. It was sterilized by autoclaving at 121 ^{0}C temperature, 15 psi pressure for 20 min. Solid media contained 1.5 - 2.0 per cent agar in mineral salts medium. One gram of each soil sample was added to each 250 ml Erlenmeyer flask containing 100 ml of sterilized mineral salt medium (MSM) to which 5 mg/L or ml/L concentration of difenoconazole, myclobutanil and fluopyram + tebuconazole were added separately. These flasks were incubated using rotary shaker cum incubator at 30 ^{0}C and 150 rpm for a period of 7 days. One ml of this 7 days old culture was transferred to sterilized flask with fresh mineral salt medium (MSM) containing 10 mg/L concentration of above mentioned fungicides and incubated for another 7 days. Further one ml of this culture was transferred to fresh MSM with 10 mg/L fungicide concentration and incubated at 30 ^{0}C and 150 rpm for another week. These procedure was followed up to 28 days.

After 28 days of incubation, one ml enriched sample were separately pipette out and diluted using serial dilution technique up to ten fold dilutions aseptically and spread on sterilized mineral salt agar medium plates containing 5 mg/L fungicides individually. The plates were incubated at 37 ^{0}C for 96 hrs. Individual colonies were streaked and re-streaked repeatedly and the pure cultures were stored at 4 ^{0}C till further experimentation.

Identification of isolates

Colony characteristics

The efficient fungicide degrading strains were characterized (identified up to generic level) based on colony characteristics and biochemical tests as detailed below. Colony characteristics of the isolates were studied according to Holt *et al*., (1994) [9]. All the tests were performed using actively growing bacterial cultures. Bacterial colonies were observed for colony morphology and Gram's staining. Bacterial cultures were grown on Nutrient agar plates to examine the colour (white, cream, yellow, creamish yellow, brown, light brown, pink); size (pinpointed, small, large, very large); shape (circular, irregular, filamentous, rhizoid); margin (entire, undulate, filiform, lobate); elevation (flat, raised, convex, pulvinate, umbonate); surface (smooth, rough, glistening, dull) and pigmentation [present (+), absent (-)] properties determined by visual observation as well as by using light microscope.

Biochemical test

Biochemical tests of the isolates were studied according to Holt *et al*., (1994) [9]. Bacterial isolates were also subjected to biochemical tests *viz*., Gram staining Amylase activity, Gelatinase activity Gelatinase activity Catalase test, Hydrolysis of starch, Casein hydrolysis, Acid and gas

production, Citrate utilization, Motility Test, Oxidase Test, Urease Test, Methyl Red Test, Voges-Proskauer (V.P.) Test, Indole Test, Urease test, Nitrate Reduction and Fermentation of carbohydrate were carried out according to Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1994) [9].

Results and Discussion

Isolation of fungicide degrading microorganisms

The soil samples were collected from grape orchards of different tehsils as well as locations of Nashik, Ahmednagar, Pune, Solapur and Sangli districts of Maharashtra during 2017 – 2018. The 17 isolates (FDB1 to FDB17) were obtained from soil samples which had ability to degrade difenoconazole, myclobutanil and fluopyram+ tebuconazole fungicides on mineral salt medium (PLATE I). The obtained fungicide degrading isolates are presented in Table 1.

Table 1: List of obtained fungicide degrading isolates from major grape growing districts in Maharashtra

Sr. No.	District	Tehsil	Location	GPS Location		Obtained isolates	Assign isolate code
				N	E		
1	Nashik	Niphad	Nandur Madhmeshwar	20.018576	74.149475	3	FDB1
							FDB2
							FDB3
		Dindori	Mavadi	20.317182	73.933401	2	FDB4
							FDB5
		Nashik	Matori	20.054277	73.737447	1	FDB6
2	Ahmednagar	Rahuri	MPKV	19.350359	74.651093	1	FDB7
		Rahata	Loni	19.583452	74.484661	1	FDB8
3	Pune	Junnar	Narayangaon	19.126099	73.968801	1	FDB9
		Ambegaon	Kalamb	19.046274	73.954312	1	FDB10
		Haveli	Manjri	18.534663	73.995161	1	FDB11
4	Solapur	Malshiras	Paniv	17.848353	74.954313	1	FDB12
		Pandharpur	Kasegaon	17.614136	75.334145	1	FDB13
5	Sangli	Walwa	Walwa	17.031158	74.380154	1	FDB14
		Tasgaon	Kumthe	16.962895	74.657097	1	FDB15
		Jath	Daphalapur	16.973460	75.097541	2	FDB16
							FDB17
Total						17	

It was observed from Table 1, the maximum number of fungicide degrading isolates were obtained from Nashik district (6 isolate) and it was followed by Sangli (4 isolate), Pune (3 isolate), Ahmednagar (2 isolate) and Solapur district (2 isolates).

Identification of isolates

All the 17 fungicide degrading isolates were subcultured on nutrient agar medium and incubated at 30 ^{0}C for 2 days. The colony characteristics of fungicide degrading isolates are given in Table 2.

Colony characteristics

Results revealed that, the slight variation among the all isolates were observed in colony characteristics *viz*., shape, margin, elevation, surface structure, consistency, opacity and pigmentation. Results indicated that, cell shape were irregular, round and uneven; margin were entire, lobed and undulate; elevation of colony were low convex, convex and flat; surface texture of colonies were smooth; consistency were watery, gummy and coarse; opacity of colonies were translucent or opaque and pigmentation such as, light red, light yellow, creamy, light green and grey white were observed in colony characteristics of these isolates.

Biochemical test

Different biochemical test were performed to characterized and identify strains up to genera on the basis of Bergy's Manual of Determinative Biology (Holt *et al*., 1994) [9]. The results of biochemical test of 17 fungicide degrading isolates are presented in Table 3.

On the basis of colony characteristics as well as biochemical test, eight Gram +ve strains and nine Gram -ve strains were identified up to generic level. The Gram +ve strains were 3 strains of *Micrococcus* spp., 2 strains of *Bacillus* spp., a strain of *Staphylococcus* spp., *Inquilinus* spp. and *Lysinibacillus* spp. In Gram –ve strains *viz*., 8 strains of *Pseudomonas* spp. and a *strenotophomonas* spp. were identified.

Results revealed that, the maximum number of isolates were obtained as *Pseudomonas* spp. All the strains were showed MR and VP test negative. In case of indole production test, only strain FDB17 (*Bacillus* spp.) was showed positive while rest of the all strains were negative to indole production test. FDB1 to FDB17 strains were for positive to catalase test.

Table 2: Colony characteristics of different fungicide degrading isolates

Sr. No.	Isolates	Shape	Margin	Elevation	Consistency	Opacity	Pigmentation
1	FDB1	Irregular	Lobed	Low convex	Watery	Translucent	Light Yellow
2	FDB2	Uneven	Entire	Flat	Gummy	Opaque	Light Yellow
3	FDB3	Round	Entire	Convex	Gummy	Opaque	White
4	FDB4	Uneven	Entire	Flat	Gummy	Opaque	Light Yellow
5	FDB5	Round	Entire	Flat	Gummy	Opaque	Creamy
6	FDB6	Irregular	Lobed	Low convex	Watery	Translucent	Light yellow
7	FDB7	Uneven	Entire	Flat	Gummy	Opaque	Light Yellow
8	FDB8	Uneven	Entire	Flat	Gummy	Opaque	White yellow
9	FDB9	Uneven	Entire	Flat	Gummy	Opaque	Light Yellow

10	FDB10	Irregular	Lobed	Low convex	Watery	Translucent	Light yellow
11	FDB11	Uneven	Entire	Flat	Gummy	Opaque	Light Yellow
12	FDB12	Uneven	Entire	Flat	Gummy	Opaque	Light Yellow
13	FDB13	Uneven	Entire	Flat	Gummy	Opaque	Light Yellow
14	FDB14	Uneven	Entire	Flat	Gummy	Opaque	Light Green
15	FDB15	Uneven	Entire	Flat	Gummy	Opaque	Light Yellow
16	FDB16	Round	Entire	Umbonate	Coarse	Opaque	Pale white
17	FDB17	Round	Undulate	Convex	Coarse	Opaque	Pale white

Table 3: Biochemical characteristics of fungicide degrading isolates

Biochemical Characterization	**Isolated Fungicide Degrading Bacteria (FDB)**																
	FDB 1	**FDB 2**	**FDB 3**	**FDB 4**	**FDB 5**	**FDB 6**	**FDB 7**	**FDB 8**	**FDB 9**	**FDB 10**	**FDB 11**	**FDB 12**	**FDB 13**	**FDB 14**	**FDB 15**	**FDB 16**	**FDB 17**
Indole test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
MR test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
Urea hydrolysis	+	+	-	+	-	+	+	+	+	+	+	+	-	-	-	+	+
Gelatin hydrolysis	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	-	-
Starch hydrolysis	+	+	-	+	-	+	+	+	+	+	+	+	-	+	+	+	+
Glucose fermentation	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-
Gas production	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Citrate	+	+	-	+	-	-	+	+	+	-	+	+	-	+	+	+	+
Casein	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
3% KOH	-	+	-	+	-	-	+	-	+	+	+	+	-	+	+	-	-
Nitrate reduction	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Gram Staining	+	-	+	-	+	+	-	+	-	+	-	-	-	-	-	+	+
Identified genera	*Micro Coccus* spp.	*Pseu dom onas* spp.	*Staph ylococc us* spp.	*Pseu dom onas* spp.	*Lysini bacill us* spp.	*Micro coccu s* spp.	*Pseu dom onas* spp.	*Inquili nus* spp.	*Pseu dom onas* spp.	*Micro coccu s* spp.	*Pseu dom onas* spp.	*Pseu dom onas* spp.	*Steno tropho monas* spp.	*Pseu dom onas* spp.	*Pseu dom onas* spp.	*Bacil lus* spp.	*Bacil llus* spp.

The findings of present investigation are also in accordance with Pawar and Mali, (2014a) [13] and Pawar and Mali, (2014b) [14] who collected the soil samples from grape rhizosphere and isolated the dichlorovos degrading strain of *Bacillus* spp. and Quinolphos degrading *Pseudomonas* spp., respectively. Results of present study are in the conformity with earlier findings of Satapute and Kaliwal, (2016) [18] that isolated the propiconazole fungicide degrading 27 *Pseudomonas aeruginosa* strains from paddy field. Salunkhe *et al*. (2015) [15] reported that profenophos, carbendazim, myclobutanil, flusilazole and tetraconazole in MSM as well as on grape berries degrade effectively by *Bacillus subtilis* strains. Yadav *et al*., (2016) reported that *Staphylococcus* spp., *Micrococcus* spp. and *Pseudomonas* spp. strains had degrading Malathion and Dichlorvos insecticides.

Results of present study are in the conformity with earlier findings of Mercadier *et al*., (1997) [11] who isolated the iprodione degrading bacteria *viz*., *Pseudomonas fluorescence*, *Pseudomonas* spp. and *Pseudomonas paucimobilis* from soil. Eizuka *et al*., (2003) [6] isolated ipconazole triazole fungicide degrading microorganisms consisting a bacterial, 12 actinomycetous and 7 fungal strains from paddy soil. Sarkar *et al*., (2009) [17] isolated propargite degrading *Pseudomonas* strains from tea rhizosphere soil on MSM. Sehnem, *et al*., (2009) [21] reported that tebuconazole biodegradation by bacteria isolated from contaminated soils. Dwivedi, *et al*., (2010) [5] isolated and characterized butachlor-catabolizing bacterial strain *Stenotrophomonas acidaminiphila* JS-1 from soil. Fang *et al*., (2010) [8] isolated the *Pseudomonas* spp. on MSM from carbendazim contaminated soil in vegetable cultivation under greenhouse. Cycon *et al*., (2011) [3] isolated *Bacillus* spp. TDS-2 strain from sandy soil previously treated with thiophanate methyl (TM) in mineral salt medium (MSM) and soil. Earlier, Youness *et al*., (2018) [24] isolated the tebuconazole degrading *Bacillus* strains and *Pseudomonas* spp. Samadi, *et al*., (2019) [16] isolated *Lysinibacillus macrolides* and *Bacillus firmus* strains from contaminated soil which had biodegrading ability of polychlorinated biphenyls.

FDB1 **FDB2** **FDB3** **FDB4**

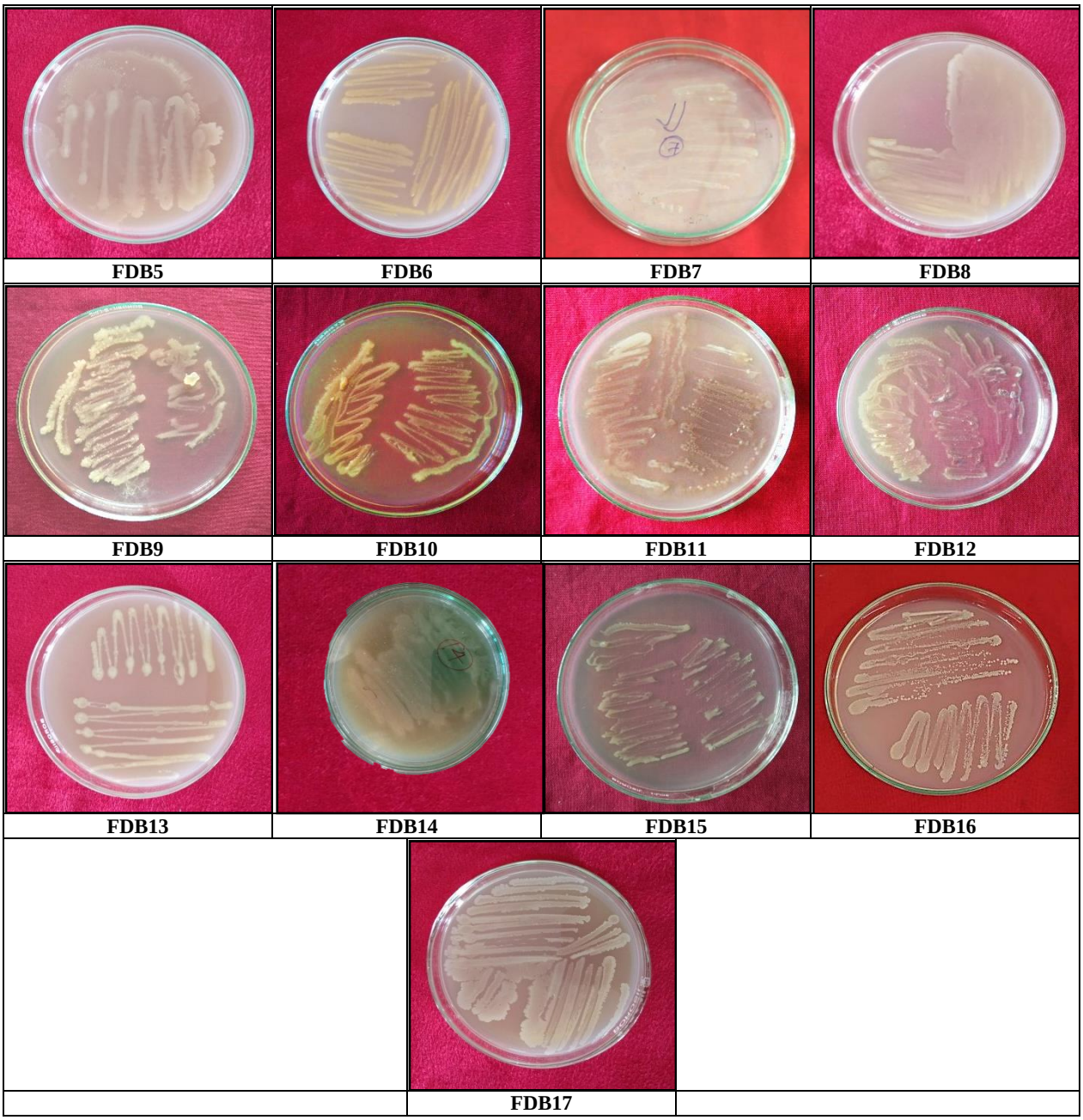

Plate 1: Growth of isolated fungicide degrading bacteria on nutrient agar medium

Conclusion

Fungicide degrading isolates (FDB1 to FDB17) were obtained from all major grape growing districts of Maharashtra. It was naturally occurring in rhizosphere soil due to better adaptability of microbes to pesticide contaminated sites over many years.

References

1. Angioni A, Aguilera Del Real A, Russo M, Melis M, Cabitza F, Cabras P. Triazole fungicide degradation in peaches in the field and in model systems. Food Additives and Contaminants 2003;20(4):368-374.
2. Arbeli Z, Fuentes CL. Microbial degradation of pesticides in tropical soils. In P. Dion (Ed.), Soil biology and agriculture in the tropics, Berlin: Springer 2010, 251-274.
3. Cycon M, Wojcik M, Piotrowska-Seget Z. Biodegradation kinetics of the benzimidazole fungicide thiophanate-methyl by bacteria isolated from loamy sand soil. Biodegradation 2011;22(3):573-583.
4. Dong X, Hong Q, He L, Jiang X, Li S. Characterization of phenol degrading bacterial strains isolated from natural soil. International Biodeterioration & Biodegradation 2008;62:257-262.
5. Dwivedi S, Singh BR, Al-Khedhairy AA, Alarifi S, Musarrat J. Isolation and characterization of butachlor-catabolizing bacterial strain *Stenotrophomonas acidaminiphila* JS-1 from soil and assessment of its biodegradation potential. Letters in Applied Microbiology 2010;51:54-60.
6. Eizuka T, Ito A, Chida T. Degradation of Ipoconazole by Microorganisms Isolated from Paddy Soil. J Pestic. Sci 2003;28(2):200-207.
7. Elmholt E. Effect of propiconazole on substrate amended soil respiration following laboratory and field application. Journal of Pesticide Science 1992;34:139-146.
8. Fang H, Wang Y, Gao C, Yan H, Dong B, Yu Y. Isolation and characterization of *Pseudomonas* spp. CBW capable of degrading carbendazim. Biodegradation 2010;21(6):939-946.

9. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of determinative bacteriology, 9th edn. The Williams and Wilkins co, Baltimore 1994.
10. Kulkarni AG, Kaliwal BB. Bioremediation of methomyl by soil isolate- *Pseudomonas aeruginosa*. Journal of Environmental Science, Toxicology and Food Technology 2014;8(12):1-10.
11. Mercadier C, Vega D, Bastide J. Iprodione degradation by isolated soil microorganisms. FEMS Microbiology Ecology 1997;23:207-215.
12. Munier LC, Borde O. Effect of a triazole fungicide on the cellulose decomposition by the soil microflora. Chemosphere 2000;41:1029-1035.
13. Pawar KR, Mali GV. Biodegradation of the organophosphorus insecticide dichlorovos by *Bacillus* species isolated from grape wine yard soils from Sangli district, M.S., India. International Research Journal of Environmental Sciences 2014a;3(5):8-12.
14. Pawar KR, Mali GV. Biodegradation of the Quinolphos insecticide by *Pseudomonas* strain from grape rhizosphere soils, International Research Journal of Environmental Sciences 2014b;3(1):606-613.
15. Salunkhe VP, Sawant IS, Banerjee K, Wadkar PN, Sawant SD. Enhanced Dissipation of Triazole and Multiclass Pesticide Residues on Grapes after Foliar Application of Grapevine-Associated *Bacillus* Species. J Agric. Food Chem 2015;63:10736-10746. DOI: 10.1021/acs.jafc.5b03429
16. Samadi A, Sharifi H, Ghobadi Nejad Z, Yaghmaei S. Biodegradation of polychlorinated biphenyls by *Lysinibacillus macrolides* and *Bacillus firmus* isolated from contaminated soil. International Journal of Engineering Transactions B: Applications 2019;32(5):628-633.
17. Sarkar S, Seenivasan S, Premkumar R. Biodegradation of propiconazole by *Pseudomonas putida* isolated from tea rhizosphere. Plant Soil Environment 2009;55:196-201.
18. Satapute P, Kaliwal B. Biodegradation of the fungicide propiconazole by Pseudomonas aeruginosa PS-4 strain isolated from a paddy soil. Annals of Microbiology 2016;66(4):1355-1365. DOI 10.1007/s13213-016-1222-6
19. Satapute PP, Olekar HS, Shetti AA, Kulkarni AG, Hiremath GB, Patagundi BI *et al*. Isolation and characterization of nitrogen fixing *Bacillus subtilis* strain as-4 from agricultural soil. International Journal of Recent Scientific Research 2012;3:762-765.
20. Sawant IS, Sawant SD. Diseases of Grapes. In: Diseases of Fruit Crops. (Eds.) Mishra AK, Chowdappa P, Sharma P, Khetarpal RK. Indian Phytopathological Society, New Delhi 2012, 113-147.
21. Sehnem NT, Souza-Cruz P, Maria Do Carmo R, Peralba, Marco A, Zachia Ayub MA. Biodegradation of tebuconazole by bacteria isolated from contaminated soils. Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes 2009;45(1):67-72.
22. Seubert NYW. Determination of isoprenoid compounds by microorganisms. Isolation and characterization of an isoprenoid degrading bacterium. *Pseudomonas citronellolis*, new species. Journal of Bacteriology 1960;79:426-434.
23. Singh DK. Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments. Indian Journal Microbiology 2008;48:35-40.
24. Youness M, Sancelme M, Combourieu B, Besse-Hoggan P. Identification of new metabolic pathways in the enantioselective fungicide tebuconazole biodegradation by *Bacillus* spp. 3B6. Journal of Hazardous Materials 2018;351:160-168.