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IDENTIFICATION OF BENEFICIAL ENDOPHYTES AGAINST BLACK ROT DISEASE IN CAULIFLOWER BY USING FAME ANALYSIS

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ABSTRACT

Black rot is a destructive disease of cauliflower through out the world caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson. The black rot pathogen is seedborne and spreads in the vascular system of the leaf and stem. In the present study Endophytic microorganisms that colonize the internal tissues of plants enhance agricultural production through plant growth promoting mechanisms. Considering the enormous potential of the endophytic bacteria, a research programme was framed to study the role of these bacteria in plant growth promotion of crop plants belonging to vegetables. The endophytic bacteria were isolated from different parts of cauliflower plants. Forty one out of the eighty eight bacterial isolates selected were Gram positive belonging to *Bacillus* sp. while forty seven were Gram negative belonging to *Citrobacter, Acinetobacter, Pseudomonas*. More than 50% of all the endophytic isolates produced P solubilization, Indole acetic acid, Acetelene reduction assay activity, Ammonia and HCN in the culture broth. After screening of all positive samples we got two bacteria colony appear highly sensitive against black rot disease with sample code PSBnR5 and PSBkS10. So we identify these samples through high end molecular technology i.e Fatty acid methyl esterase (FAME) analysis technology. By using FAME analysis was used and isolate PSBnR5 had similarity index 0.54 which confirmed as *Bacillus subtilis* and isolate PSBkS10 were belonged to*Pseudomonas fluorescens* with similarity index 0.75.

KEYWORDS : Endophytes , cauliflower, FAME

Introduction

Cauliflower (*Brassica oleracea*) ia annual plant and one of the widely consumed vegetable of several, in the family *Brassicaceae*. It has a remarkable nutritional values as it contains high dietary fiber, vitamin C as well as many phytochemicals (carotenoids, glucosinolates and Indole-3-carbinol), antioxidants and anticancer compound such as sulfonates (Alvarez 2000).

The cauliflower crop is imperiled to attack by a variety of phytopathogens by both fungi and bacteria during nursery as well as in field conditions. Among various diseases black rot, being caused by bacterium *Xanthomonas campestris*pv *campestris* is responsible for considerable loss in crop yield, Along with cauliflower some other crucifers e.g mustard, cabbage, radish are also vulnerable to this disease Jemson *et al*. (2010).

Due to extensive use of pesticides on various food crops includes vegetables is leading threat to food safety and ecological balance, In this consequence it is needful to find eco-friendly. The most appropriate combinations of chemical fertilizer and biological components can contribute to enhanced crop production, Suresh babu *et al*.(2016) and are economically viable and environmentally sustainable.

Dey *et al*.(2004) reported role of PGPR in seed emergence, growth and crop yield. The main aim of biotechnological development based on PGPR has been to develop soil inoculants that can contribute to sustainable agriculture, there by diminishing the need for use of chemical fertilizers and pesticides (Adesemoye and Kloepper, 2009).

The *Pseudomonas* sp. and *Bacillus* sp. genus are mostly found in consortia of rhizosphere colonizing bacteria due to their fast growth rate and adaptable to different soil nutrients. These both groups are reported to have significant disease control capabilities for range of plant pathogens due to production of several antifungal and antibacterial bioactive molecules Nihorimbere *et al*.(2011). Many *Pseudomonas* sp. and *Bacillus* sp are also scale up to industrial level as most successful plant growth promoting and potential biocontrol agents.In the present study, We undertook two potential isolates *Pseudomonas fluorescens* PSBkS10 and *Bacillus subtilis* PSBnR5 and further proceed with our main aim to evaluate antagonism for *Xanthomonas campestris* ; Role in eliciting plant growth and nutrient management or crosstalk.

Material and Methods

Growth and maintenance of organisms used in this study

The strains PSBkS10 and PSBnR5 isolated from cauliflower sample of the Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi. X. campestris pv. campestris strains Xcc- C1 was isolated from black rot infected cauliflower at, IARI Research Farm, New Delhi by using standard procedure of isolation. All bacterial strains were maintained at 4^{0} C in YDC medium.

Identification of Microorganisms Using Fatty Acid Methyl

Young pure cultures were grown on TSB agar for 24 or 48 h at 28^{3}C. Strains that could not grow under these conditions were either grown for a longer time (72 or 96 h) or on Marine broth (Difco) with 1.5% Bacto-agar (Difco). The FAME profiles of the latter were used for clustering but not for identification. A quantitative analysis of cellular fatty acid compositions was further performed, using the gas-liquid chromatographic procedure as previously described (Mergaert et al 1993) he resulting profiles were identified with the Microbial Identification software (MIDI) using the TSBA database (version 4.0) (Microbial ID, Newark, DE, USA). For clustering, the data were transferred to BioNumerics software (Applied Maths, Kortrijk, Belgium) and compared by UPGMA of the Canberra metric coefficients calculated between the FAME profiles. To test the reliability of the cluster identifications, the FAME profiles were also compared to several reference profiles from the TSBA4.0 library, by UPGMA of the Euclidean distance coefficients. For representative pure cultures of each cluster, cell morphology, Gram stain and spore formation was recorded.

Result and discussion

After screening of all positive samples we got two bacteria colony appear highly sensitive against black rot disease with sample code PSBnR5 and PSBkS10. So we identify these samples through high end molecular technology I.e. Fatty acid methyl esterase (FAME) Analysis technology. After identification I.e. FAME analysis we got same result with both sample id. Sample id PSBnR5 confirm as *Bacillus subtilis* with similarity index 0.54 and sample id PSBkS10 Confirm as Pseudomonas fluoresceins with similarity index 0.75 .Both samples id result confirm as following.

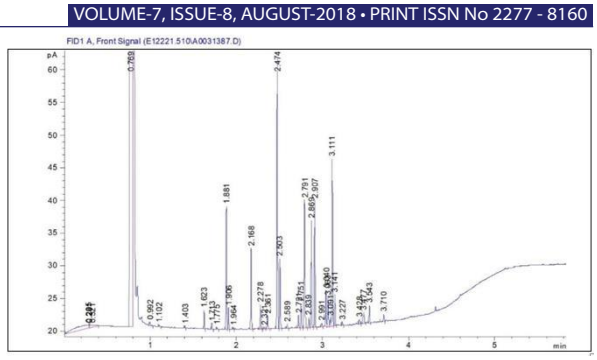
Bacillus FAME Result
Volume DATA File:E122215.10A SampCtr:3 ID
Type:Samp Bottle:2 Method:RTSBA6 Number:1387
Created :11/21/2016 12:29:52 PM
Sample ID:Sample No.1T1 (PSBnR5)

RT	Respo	Ar/	RFa	ECL	Peak Name	Perc	Comment1	Comment2
0.28	15383	0.1	----	3.51		----	< min rt	
0.29	413	0.0	----	3.54		----	< min rt	
0.32	3291	0.0	----	3.74		----	< min rt	
0.76	1.075	0.0	----	6.65	SOLVENT	----	< min rt	
0.99	1142	0.0	----	8.11		----	< min rt	
1.10	341	0.0	----	8.82		----	< min rt	
1.40	423	0.0	1.11	10.6	11:0 iso	0.21	ECL deviates	Reference -
1.62	3035	0.0	1.06	11.6	12:0 iso	1.42	ECL deviates	Reference -
1.71	1032	0.0	1.05	11.9	12:0	0.47	ECL deviates	Reference -
1.77	332	0.0	----	12.2		----		
1.88	20338	0.0	1.02	12.6	13:0 iso	9.14	ECL deviates	Reference -
1.90	3548	0.0	1.02	12.7	13:0 anteiso	1.59	ECL deviates	Reference
1.96	341	0.0	----	12.9		----		
2.16	13373	0.0	0.99	13.6	14:0 iso	5.82	ECL deviates	Reference -
2.27	4288	0.0	0.98	13.9	14:0	1.85	ECL deviates	Reference -
2.32	380	0.0	0.98	14.1	13:0 iso	0.16	ECL deviates	
2.36	2476	0.0	----	14.2	unknown	----	ECL deviates	
2.47	46068	0.0	0.96	14.6	15:0 iso	19.5	ECL deviates	Reference -
2.50	11802	0.0	0.96	14.7	15:0 anteiso	4.99	ECL deviates	Reference -
2.58	768	0.0	----	15.0	15:0	----	ECL deviates	
2.72	2331	0.0	0.95	15.4	16:1 w7c	0.97	ECL deviates	
2.75	4337	0.0	0.95	15.5	Sum In	1.80	ECL deviates	14:0
2.79	23988	0.0	0.94	15.6	16:0 iso	9.96	ECL deviates	Reference -
2.83	1842	0.0	0.94	15.7	16:1 w11c	0.76	ECL deviates	
2.86	19334	0.0	0.94	15.8	Sum In	8.00	ECL deviates	16:1
2.90	21740	0.0	0.94	15.9	16:0	8.98	ECL deviates	Reference -
2.99	694	0.0	0.94	16.2	15:0 2OH	0.29	ECL deviates	
3.04	6654	0.0	0.93	16.4	17:1 iso	2.73	ECL deviates	
3.06	5997	0.0	0.93	16.4	17:1 iso	2.46	ECL deviates	
3.09	1555	0.0	0.93	16.5	17:1 anteiso	0.64	ECL deviates	
3.11	29965	0.0	0.93	16.6	17:0 iso	12.2	ECL deviates	Reference -
3.14	5759	0.0	0.93	16.7	17:0 anteiso	2.36	ECL deviates	Reference -
3.22	762	0.0	0.93	17.0	17:0	0.31	ECL deviates	Reference -
3.42	1044	0.0	0.92	17.6	18:0 iso	0.42	ECL deviates	Reference -
3.47	3743	0.0	0.92	17.7	18:1 w9c	1.52	ECL deviates	
3.54	3370	0.0	0.92	17.9	18:0	1.36	ECL deviates	Reference -
3.71	1635	0.0	----	18.5		----		
----	4337	---	----	----	Summed	1.80	12:0	Unknown
----	-----	---	----	----		----	16:1 iso I/14:0	14:0
----	19334	---	----	----	Summed	8.00	16:1 w7c/16:1	16:1

ECL Deviation: 0.002 **Reference ECL Shift: 0.004**
Number Reference Peaks: 16
Total Response: 239711 **Total Named: 237402**
Percent Named: 99.04% **Total Amount: 228478**

Matches:

Library	Sim	Entry Name
RTSBA 6	0.536	Bacillus-subtilis
	0.406	Bacillus-cereus-GC subgroup A



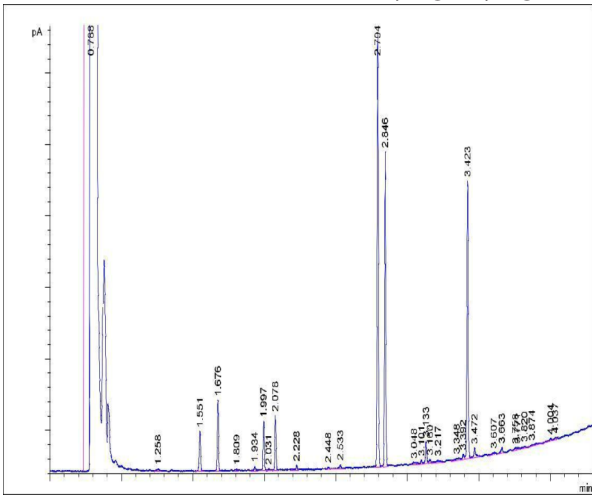
Volume DATA	File:E158315.10A	SampCtr:27	ID
Type:Samp	Bottle:19	Method:RTSBA6	Number:1294
Created :11/21/2016 3:54:2 PM			
Sample ID:Sample No.T2 (PSBkS10)			

RT	Respo	Ar/	RFa	ECL	Peak Name	Perc	Comment1	Comment2
0.78	7.226	0.0	----	6.74	SOLVENT	----	< min rt	
1.25	946	0.0	----	9.97		----		
1.55	7684	0.0	1.07	11.4	10:0 3OH	3.95	ECL deviates	
1.67	12368	0.0	1.04	12.0	12:0	6.16	ECL deviates	Reference
1.80	591	0.0	----	12.5	unknown	----	ECL deviates	
1.93	1043	0.0	----	12.9		----		
1.99	8243	0.0	0.98	13.2	12:0 2OH	3.88	ECL deviates	
2.03	527	0.0	0.97	13.3	12:1 3OH	0.25	ECL deviates	
2.07	8976	0.0	0.97	13.4	12:0 3OH	4.18	ECL deviates	
2.22	1271	0.0	0.95	14.0	14:0	0.58	ECL deviates	Reference -
2.44	480	0.0	0.94	14.7	15:0 anteiso	0.22	ECL deviates	Reference -
2.53	1393	0.0	----	15.0	15:0	----	ECL deviates	
2.79	71448	0.0	0.92	15.8	Sum In	31.6	ECL deviates	16:1
2.84	51736	0.0	0.92	15.9	16:0	22.8	ECL deviates	Reference -
3.04	763	0.0	0.92	16.6	17:0 iso	0.34	ECL deviates	Reference
3.10	1202	0.0	0.92	16.8	17:1 w8c	0.53	ECL deviates	
3.13	4325	0.0	0.92	16.9	17:0 cyclo	1.91	ECL deviates	
3.16	1059	0.0	0.92	16.9	17:0	0.47	ECL deviates	Reference -
3.21	1882	0.0	0.92	17.1	16:0 iso 3OH	----	> max ar/ht	
3.34	1512	0.0	0.92	17.6	18:3 w6c	----	> max ar/ht	
3.39	1107	0.0	----	17.7		----		
3.42	48312	0.0	0.92	17.8	Sum In	21.3	ECL deviates	18:1 w7c
3.47	2747	0.0	0.92	17.9	18:0	1.21	ECL deviates	Reference -
3.60	598	0.0	----	18.4		----		
3.66	1478	0.0	----	18.6		----		
3.75	561	0.0	0.92	18.9	19:0 cyclo	0.25	ECL deviates	
3.77	731	0.0	----	18.9		----		
3.82	602	0.0	0.92	19.1	18:1 2OH	0.27	ECL deviates	
3.87	671	0.0	----	19.3		----		
4.00	1573	0.0	----	19.7		----	> max ar/ht	
4.03	555	0.0	----	19.8		----		
----	71448	---	----	----	Summed	31.6	16:1	16:1
----	48312	---	----	----	Summed	21.3	18:1 w7c	18:1 w6c

ECL Deviation: 0.004 **Reference ECL Shift: 0.003**
Number Reference Peaks: 7
Total Response: 234400 **Total Named: 222304**
Percent Named: 94.84% **Total Amount: 212020**

Matches:

Library	Sim	Entry Name
RTSBA6	0.733	Pseudomonas-fluorescens
	0.634	Pseudomonas-mucidolens
	0.631	Pseudomonas-savastanoi-oleae
	0.610	Pseudomonas-syringae-syringae



Conclusion

From the above study it is concluded that Sample id PSBnR5 confirm as *Bacillus subtilis* with similarity index 0.54 and sample id PSBkS10 Confirm as *Pseudomonas fluoresceins* may help in suppressing black rot disease pathogen ultimately cauliflower production will increase.

References

1. Adesemoye, A. O., Kloepper, J. W. (2009). Plant–microbes interactions in enhanced fertilizer-use efficiency.Applied Microbiology and Biotechnology, 85: 1-12.
2. Alvarez, A. (2000). Black rot of crucifers. In: Mechanisms of resistance to plant dis eases (A. Slusarenko, R. S. S. Fraser and L. C. van Loon, Eds.). Dordrecht: Kluwer Academic publishers. Pp.21-52.
3. Dey, R., Pal, K. K., Bhatt, D. M., Chauhan, S. M. (2004). Growth promotion and yield enhancement of peanut (Arachishypogaea L.) by application of plant growth-promoting rhizobacteria. Microbiological Research, 159: 371-394.
4. Jensen, B.D., Vicente J.G. Manandhar H.K. and Roberts S.J. (2010). Occurrence and diversity of Xanthomonascampestrispv. campestris in vegetable Brassica fields in Nepal. Plant Dis. 94: 298-305. Jorgensen, M.C. and Walter J.M. (1955).The 1953 outbreak of black rot at Ruskin. Proc. Fla. Hort.
5. Mergaert, J., Verdonck, L. and Kersters, K. (1993) Transfer of Erwinia ananas (synonym, Erwinia uredovora) and Erwinia stewartii to the genus Pantoea emend. as Pantoea ananas (Serrano 1928) comb. nov. and Pantoea stewartii (Smith 1898) comb. nov., respectively, and description of Pantoea stewartii subsp. indologenes subsp. nov.. Int. J. Syst. Bacteriol. 43, 162^ 173.
6. Nihorimbere, V., Ongena,M. Smargiassi,M., Thonart, P. (2011).Beneficial effect of the rhizosphere microbial community for plant growth and health.Biotechnol.Agron. Soc. Environ. 15: 327-337.
7. Sureshbabu, K., Amaresan, N., Kumar, K. (2016). Amazing multiple function properties of plant growth promoting rhizobacteria in the rhizosphere soilInternatinal Journal Current Microbiological Applied Science, 5 : 661-683.