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Numerical Assessment of Mycelium Color in Classification of Some *Streptomyces* Isolates

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ABSTRACT

A number of 15 *Streptomyces* isolates with white, green and blue colored aerial mycelium were isolated from soil. Three isolates (w 01, w 02 & w 03), were identified as belonging to *S. aureomonopodiales*, 2 isolates (w 04, w 05) as belonging to *S. aureocirculatus*, 2 isolates (g 14, g 15) as strains of *S. hirsutus*, 3 isolates (g 06, g 07 & g 08) as strains of *S. prasinus*, one isolate (b 09) as belonging to *S. Lanatus*, 2 isolates (b 10, b 11) as belonging to *S. indigocolor* and 2 isolates (b 12 & b 13) as strains of *S. amakusaensis*. For each isolate, 53 units character were numerically coded for computer analysis.Canberra distance (CD) and flexible sorting method (G) were applied for calculation of mutual relationships and clustering of the isolates (OTU's). Results of numerical analysis assessed the color of aerial mycelium as strong phenotypic character in classification of the genus *Streptomyce*s.

Key Words: Numerical taxonomy; *Streptomyces*; Mycelium color

INTRODUCTION

An extensive literature review concerning the taxonomic status of the species of the genus *Streptomyces* has been made on the base of classical microbiological and chemo taxonomical methods (Christova *et al*., 1995). Silvestri *et al*. (1962) proved that the result of the analysis of 200 strains for 100 characters, grouped them to 25 variant groups. The analysis showed that many of the characteristics used for the classification of *Streptomyces* species are strongly variable and hard for interpretation. A considerable step ahead is the numerical classification of Williams *et al*. (1983), which used 475 strains among them 394 *Streptomyces* type cultures from ISP and other 14 *actinomycete* genera. Ochi (1992) proved the efficiency of r-protein analysis as a novel approach for taxonomy of 11 *streptomyces* strains by using numerical methods. Bouchek-Mechiche *et al*. (1998) reported that phenotypic characteristics and numerical analysis clearly differentiated all the 31 *streptomyces* strains isolated from common and netted scabs in France. Doumbou *et al*. (2001) applied numerical taxonomy to compare 16 non-pathogenic actinomycetes isolated from common scab lesion on potato tuber with *Streptomyces scabiei*, they reported that the use of phenotypic traits to differentiate pathogenic streptomycetes from non-pathogenic ones is difficult; in contrast none of the non-pathogenic isolates could be confused with *Streptomyces scabiei* in regard to 16S r-DNA sequence. Trujillo and Goodfellow (2003) used numerical taxonomic data to generate a frequency matrix designed to facilitate the identification of clinically significant *Actinomadura*, *Nocardiopsis* and *Streptomyces* stains to the species level.

The aim of the present study is the use of numerical taxonomy to assess the color of mycelium as a phenotypic trait in the classification of streptomycetes.

MATERIALS AND METHODS

Streptomycetes with white, green and blue colored aerial mycelia were isolated from soil samples according to the method described by Wollum (1982). The samples were collected from different localities at Dakahlyia governorate, Egypt. For each isolates the state of growth, colony color (averse, reverse) and sporulation using malt yeast extract agar and inorganic salts starch agar media, production of melanoid pigments and soluble colors other than melanoids, nitrate reduction, starch hydrolysis, production of H_2S, decomposition of cellulose, liquefaction of gelatin, peptonization and coagulation of milk, utilization of different nitrogen and olive oil sources, hydrolysis of urea and citrate utilization were carried out according to the methods described by American Public Health Association, APHA (1998).

The isolates were identified on the basis of the description of *Streptomyces* species in the articles of ISP (Shirling & Gottlieb, 1966), the key of Pridham and Tresner (1974) and that of Bregey's manual of determinative bacteriology (Williams *et al*., 1989). All descriptions (units character) were converted into digits and coded by either Zero (means negative or absent) or one (means positive or present). The names of character, character state and coding are given in Table I. Mutual relationships between isolates (OTU's) were estimated by applying the Canberra distance coefficient (CD) and the classification dendrogram was created by the flexible sorting method (G) of cluster

Table I. Names of characters, units character and their coding

No	Characters	Units character	Type	Coding
1	Growth on malt yeast extract agar (3)	1. Good	Bi-state	0, 1
		2. Moderate	Bi-state	0, 1
		3. Scant	Bi.state	0, 1
2	Growth on inorganic salts starch agar (3)	4. Good	Bi-state	0, 1
		5. Moderate	Bi-state	0, 1
		6. Scant	Bi-state	0, 1
3	Colony color, averse on M.Y.E. agar (5)	7. White	Bi-state	0, 1
		8. Light-green	Bi-state	0, 1
		9. Green	Bi-state	0, 1
		10. Grayish-blue	Bi-state	0, 1
		11. Sky-blue	Bi-state	0, 1
4	Colony color, reverse on M.Y.E. agar (7)	12. Grayish-yellow	Bi-state	0, 1
		13. Yellowish-pale brown	Bi-state	0, 1
		14. Grayish- yellow	Bi-state	0, 1
		15. Grayish-blue	Bi-state	0, 1
		16. blue	Bi-state	0, 1
		17. Pale yellow	Bi-state	0, 1
		18. no color	Bi-state	0, 1
5	Colony color, averse on I.S.S. agar (5)	19. White	Bi-state	0, 1
		20. Light-green	Bi-state	0, 1
		21. green	Bi-state	0, 1
		22. Grayish-blue	Bi-state	0, 1
		23. Sky-blue	Bi-state	0, 1
6	Colony color, reverse on I.S.S. agar (7)	24. Grayish-yellow	Bi-state	0, 1
		25. Yellowish-pale brown	Bi-state	0, 1
		26. Grayish- yellow	Bi-state	0, 1
		27. Grayish-blue	Bi-state	0, 1
		28. blue	Bi-state	0, 1
		29. Pale yellow	Bi-state	0, 1
		30. no color	Bi-state	0, 1
7	Pigments formation (Milaniods) (1)	31. Pigments formation (Milaniods)	Bi-state	0, 1
8	Morphology of spore chain (2)	32. Section Spirals	Bi-state	0, 1
		33. Section rectiflexibles	Bi-state	0, 1
9	Spore surface (2)	34. Smooth	Bi-state	0, 1
		35. Spiny	Bi-state	0, 1
10	Reduction of nitrate (1)	36. Reduction of nitrate	Bi-state	0, 1
11	Hydrolysis of starch (1)	37. Hydrolysis of starch	Bi-state	0, 1
12	Production of H_2S (1)	38. Production of H_2S	Bi-state	0, 1
13	Decomposition of Cellulose (1)	39. Decomposition of Cellulose	Bi-state	0, 1
14	Liquefaction of gelatin (1)	40. Liquefaction of gelatin	Bi-state	0, 1
15	Peptonization of milk (1)	41. Peptonization of milk	Bi-state	0, 1
16	Coagulation of milk (1)	42. Coagulation of milk	Bi-state	0, 1
17	Utilization of citrate (1)	43. Utilization of citrate	Bi-state	0, 1
18	Utilization of amm. nitrate (1)	44. Utilization of amm. nitrate	Bi-state	0, 1
19	Utilization of amm. nitrite (1)	45. Utilization of amm. nitrite	Bi-state	0, 1
20	Utilization of amm. chloride (1)	46. Utilization of amm. chloride	Bi-state	0, 1
21	Utilization of pot. nitrate (1)	47. Utilization of pot. nitrate	Bi-state	0, 1
22	Utilization of pot. nitrite (1)	48. Utilization of pot. nitrite	Bi-state	0, 1
23	Utilization of sod. nitrate (1)	49. Utilization of sod. nitrate	Bi-state	0, 1
24	Utilization of sod. nitrite (1)	50. Utilization of sod. nitrite	Bi-state	0, 1
25	Utilization of urea (1)	51. Utilization of urea	Bi-state	0, 1
26	Utilization of peptone (1)	52. Utilization of peptone	Bi-state	0, 1
27	Utilization of olive oil (1)	53. Utilization of olive oil	Bi-state	0, 1

analysis. All numerical calculations were performed by "Quant" program (Ismail & Batko, 1996) running on IBM compatible computer.

RESULTS

Identification. The results of cultural and biological characteristics (Table II) proved that:

1. Five isolates showing white colored aerial mycelium were reported. Among them, 3 isolates (w 01, w 02 & w 03) were identified as belonging to *S. aureomonopodiales* and 2 isolates (w 04, w 05) as belonging to *S. aureocirculatus.*

2. Five isolates with green colored aerial mycelium were detected. Among them 2 isolates (g 14, g 15) were identified as strains of *S. hirsutus* and 3 isolates (g 06, g 07 & g 08) as strains of *S. prasinus.*

3. Five *Streptomyces* isolates showing blue colored mycelium were recorded. Among them one isolate (b 09) was identified as belonging to *S. Lanatus*, 2 isolates (b 10, b 11) as belonging to *S. indigocolor* and 2 isolates (b 12 & b

Table II. Cultural and biological characteristics of *Streptomyces* isolates coded by 0 or 1(data matrix)

Ch./ otu's	W 01	W 02	W 03	W 04	W 05	G 06	G 07	G 08	B 09	B 10	B 11	B 12	B 13	G 14	G 15
1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1
2	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
4	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0
5	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
9	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0
11	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
12	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
17	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
19	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
26	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0
29	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	1	1	1	0	0	0	0	0	1	1	1	1	1	0	0
32	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
34	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
38	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
39	1	1	1	0	0	0	0	0	1	0	0	1	1	1	1
40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
41	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
42	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1
43		1	1	0	0	1	1	0	0	1	1	0	0	0	0
44	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
45	0	0	0			1	1	1	1	1	1	0	0	0	0
46	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
47	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
48	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0
49	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
50	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0
51	1	1	1	0	0	1	1	0	0	1	1	1	1	1	1
52	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
53	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0
Tot.	22	23	23	20	20	23	23	22	24	25	24	21	21	20	20

13) as strains of *S. amakusaensis.*

Numerical analysis. Results of computer analysis (Dend. 1) proved that the white series isolates were clustered as follow:

1. Two isolates of *S. aureomonopodiales* (w 02, w 03) was jointed at distance level of 2.8 (similarity level of 97.2) one isolates (w 01) of the same species was added to this group at distance level of 4.05 (similarity level of 95.95) to form sub cluster A.

2. Two isolates of *S. aureocirculatus* (w 04, w 05) were jointed at distance level of 2.8 (similarity level of 97.2) to form sub cluster B.

3. The subcultures A and B were grouped in one major cluster consisted of all the white series isolates (w 01, w 02, w 03, w 04, w 05) at distance level of 17.53 (similarity level of 82.47).

For the green series isolates, results showed that:

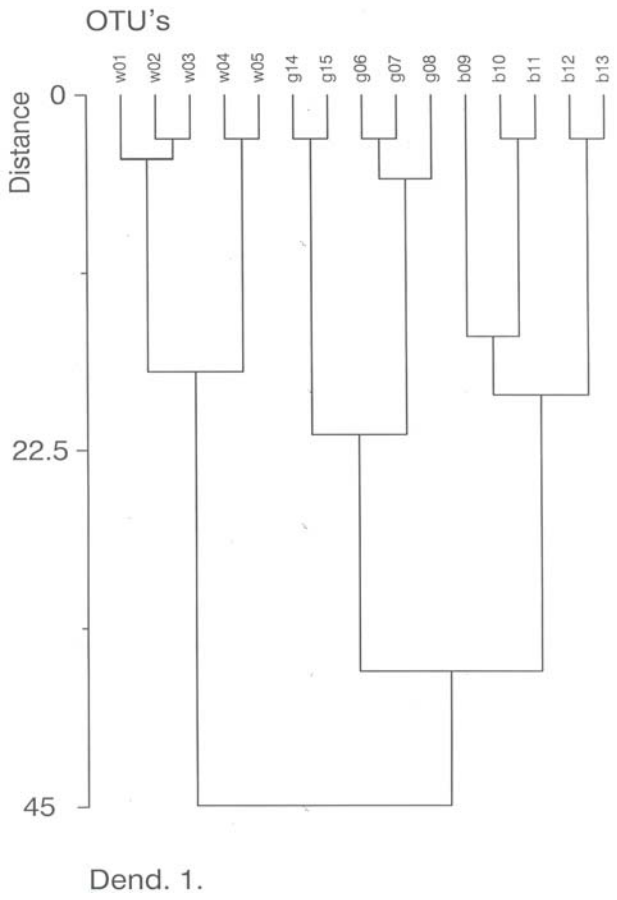
4. Two isolates of *S. hirsutus* (g 14, g 15) were grouped at distance level of 2.8 (similarity level of 97.2) to form sub cluster C.

5. Three isolates of *S. prasinus* (g 06, g 07, g 08) were jointed in one group at distance level of 2.8 and 5.3, respectively (similarity level of 97.2 & 94.7) to form sub cluster D.

6. The subcultures C and D were grouped in one major cluster consisted of all the green series isolates (g 14, g 15, g 06, g 07, g 08) at distance level of 21.51 (similarity level of 78.49).

In regard to the blue series isolates results proved that:

Dend. 1. Numerical classification of *Streptomyces* isolates on basis of Canberra distance and flexible sorting method



Dend. 1.

7. Two isolates of *S. indigocolor* (b 10, b 11) were jointed at distance level of 2.8 (similarity level of 97.2).One more isolates of *S. Lanatus* (b 09) was added to this group at distance level of 15.3 (similarity level of 84.7) to form sub cluster E.

8. Two isolates (b 12, b 13) of *S. amakusaensis* were grouped at distance level of 2.8 (similarity level of 97.2) to form sub cluster F.

9. The two sub clusters E and F were clustered in one major group consists of the all isolates with blue colored aerial mycelium (b 09, b 10, b 11, b 12, b 13) at distance level of 19.01 (similarity level of 80.99).

On focus the degree of similarity between the three major white, blue and green groups were 82.47, 80.99 and 78.49, receptively. Also, results Proved that the blue isolates were more similar to the green ones (similarity level of 63.56) than to the white series isolates (similarity level of 55.1).

Basing on this result the author strongly recommended the use of numerical methods to get better insight on to the *Streptomyces* classification. Goodfellow *et al*. (1992) stated that numerical taxonomy is of proven value both for the circumscription and identification of *Streptomyces* species. Mohamed *et al.* (2005) stated that the application of a suggested numerical taxonomy on 14 known *Streptomyces* species revealed that these species fell in to 3 major clusters based on their color of aerial mycelia. Mohamed and Galal (2005) Stated that 3 *Streptomyces* isolates, which belonged to three different series color groups (yellow, grey & red) were fall in 3 major clusters. Zhao *et al.* (2006) stated that the dendrogram constructed using un-supervised cluster analysis of the Fourier transform infrared (FT-IR) spectroscopy data was in good congruence with the four color groups and the neighbour-joining phylogenetic tree for 16S r-DNA sequencing.

The main conclusion of the present study is the assessment of aerial mycelium color as strong phenotypic trait in *Streptomyces* classification and evaluation of numerical taxonomy as a powerful tool in finding the mutual relationships between streptomycetes series with aerial mycelium of different colors.

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REFERENCES

American Public Health Association (APHA), 1998. *Standard Methods for the Examination of Water and Wastewater, 20th Edition, Chapter 5*, Washington, DC USA

Bouchek-Mcchiche, K., C. Guerin, B. Jouan and L. Gardan, 1998. *Streptomyces* species isolated from potato scabs in France: 'numerical' analysis of "biotype-100" carbon source assimilation data. *Res. Microbiol.*, 149: 653–63

Christova, K., Z. Sholeva and V. Chipeva, 1995. Application of molecular biological methods taxonomy of genus *Streptomyces*. *J. Culture Collect*, 1: 3–10

Doumbou, C., V. Akimov, M. Cote, P. Charest and C. Beaulieu, 2001. Taxonomic study on non-pathogenic streptomycetes isolated from common scab lesions on potato tubers. *System Appl. Microbiol.*, 24: 451–6

Goodfellow, M., E.V. Ferguson and J. Sanglier, 1992. Numerical classification and identification of *Streptomyces* species. *Gene*, 115: 225–33

Ismail, A. and A. Batko, 1996. A Phenetic numerical taxonomy programs in Basic. *The 1st Conference on the Role of Science in the Development of Egyptian Society and Enviroment*, Pp: 21–3. October, 1996, Benha, Egypt

Mohamed, S.H. and A.M. Galal, 2005. Identification and antiviral activities of some halotolerant streptomycetes isolated from Qaroon Lake. *Int. J. Agric. Biol.*, 5: 747–53

Mohamed, S.H., E.A. Saieh and M.M. Zaki, 2005. Identification of eight halotolerant streptomycete isolates using a suggested numerical taxonomy. *Arab University J. Agric. Sci.*, 1: 641–68

Ochi, K., 1992. Polyacrylamide gel electrophoresis analysis of ribosomal protein: a new approach for actinomycete taxonomy. *Gene*, 115: 261–5

Pridham, T.G. and H.D. Tresner, 1974. Family Streptomycetacae. *In: Bergey's Manual of Determinative Bacteriology*, Pp: 751–826. Williams and Wilkins, Baltmore, USA

Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bact*., 16: 313–40

Silvestri, L., M. Turri, L.R. Hill, E. Gilardi, 1962. A quantitative approach to the systematics of actinomycetes based on overall similarity. *In*: Ainsworth, G.C. and P.H.A. Sneath (eds.), *Microbial Classification*, Pp: 333–60. Cam-bridge: Cambridge University Press

Trujillo, M.E. and M. Goodfellow, 2003. Numerical phenetic classification of clinically significant aerobic sporoactinomycetes and related organisms. *Antonie-van-Leeuwenhoek*, 84: 39–68

Williams, S.T., M. Goodfellow, G. Alderson, E.M. Wellington, P.H. Sneath and M.J. Sookin, 1983. Numerical classification of *Streptomyces* and related genera. *Gen. J. Microbiol.*, 129: 1743–813

Williams, S.T., M. Goodfellow and G. Alderson, 1989. Genus *Streptomyces*. *In*: Williams, S.T., M.E.Sharpe and J.P. Holt (eds.), *Bregey's Manual of Determinative Bacteriology*, Pp: 2452–92. Williams and Wilkins, Baltimore, USA

Wollum, A.G., 1982. Cultural Methods for Soil Microorganisms. *In*: page, A.L. *et al*. (ed.), *Methods of Soil Analysis, Part 2, (2nd ed.)*, Pp: 781–802. Agron. Monogr. 9 ASA and SSSA, Madison

Zhao, H, R.L. Parry, D.I. Ellis, G.W. Griffith and R. Goodacre, 2006. The rapid differentiation of streptomyces isolates using fourier transform infrared spectroscopy. *Vibrational Spectrnscopy*, 40: 213–8

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