Phenol Oxydase Activity of Streptomyces from a Recultivated Dump Area

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Chromogenous Streptomyces species are capable of forming dark-coloured humic-like compounds. This property is in close connection with the formation of such phenol oxydase enzymes which are capable of catalysing the quinoid transformation of the aromatic compounds.

Substrates, mono- and diphenols required for phenol oxydase reaction can be synthetised in the metabolic processes of microorganisms /Streptomycetes/, but can also originate from any external source, e. g. from the degradation of lignine. Streptomycetes forming dark-brown pigments and humic-like compounds play an important role in soil forming processes during the recultivation of dump areas.

Materials and methods

The phenol oxydase enzyme synthesis of Streptomyces strains, isolated and identified from the original, fertile chernozem brown forest soil of Visonta and from treated samples /control and NPK + lignite variants/ of a recultivation experiment set up with Pannonian clay dumps has been investigated. Based on diagnostic data, the investigated Steptomyces strains were identified according to the taxonomic key of SZABÓ et al. /1975/. The diagnostic data thus obtained were compared with the standard type culture descriptions published by SHIRLING and GOTTLIEB /1968a; 1968b, 1969, 1972/.

Investigations were carried out in the presence of specific substrates, mono- and diphenols; D-tyrosine, p-cresol, quinol and catechol, according to KÜSTER's /1953/ phenol-oxydase test.

Plate-cultures with modified oat agar were prepared with the following composition: oat-flake = 20 g, agar = 18 g, glucose = 10 g, yeast extract = = 5 g, tapwater = 1000 cm^3, pH = 6.8.

Streptomyces strains on plate-cultures, prepared in Petri-dishes, were incubated with this medium at 28 °C for a fortnight. Following incubation, agar plate discs of 8 mm diameter were cut out from the Streptomyces colonies, and were placed on agar plates containing phenol compounds, in four replications. Composition of the phenol medium was: concentration of M/30, pH = 6.5, phosphate buffer = 1000 cm^3, agar = 16 g, phenol compound = 0.10%, D-tyrosine = 0.05%. Subsequently, the Petri-dishes were placed into a thermostat at

Table 1

The phenol oxidase reaction of the Streptomyces strains originating from the undisturbed topsoil /Chernozem brown forest/

Designation of investigated strains		Qui-nol	Catec-hol	Designation of investigated strains		Qui-nol	Catec-hol
S. galbus	F-34	–	+	S. capreolus	F-98	–	+
	F-129	–	+	S. diastatochro-			
S. endus	F-28	–	+	mogenes	F-25	–	+
	F-48	–	–	/?/	F-159	–	+
	F-57	–	–	S. filamentosus	F-144	–	+
	F-70	–	–		F-148	+	++
	F-123	–	–	S. flavofungini	F-151	–	+
	F-125	–	–	S. galileus	F-102	+	++
	F-149	–	–	S. gardneri	F-72	+	+
A. janthinus	F-66	+	+++	S. gelaticus	F-10	–	–
S. massasporeus	F-22	–	–	S. griseolus	F-126	–	–
	F-69	–	+	S. griseoplanus	F-114	+	+
	F-127/a	+	–	S. lividans	F-49	–	+
	F-127/b	–	–		F-104	–	+
S. nigrifaciens	F-40	–	–	A. longisporus	F-89	++	+++
	F-71	–	–		F-112	+	+
	F-75	–	–	S. macrosporeus	F-106	–	+
S. aburaviensis	F-90	–	+		F-124	–	+
	F-96	–	+	S. microflavus	F-121	–	+
	F-139	–	+		F-122	–	++
S. actuosus	F-55	–	+	S. niveus	F-54	+	+
S. albaduncus	F-115	–	+	S. parvullus	F-38	–	++
S. albofaciens	F-4	–	–		F-26	–	+
	F-11	–	–		F-79	–	+
	F-157	–	+		F-130	–	+
	F-163	–	–		F-141	–	+
S. alboniger	F-65	–	++		F-143	–	+
S. antimycoticus	F-3	–	+	S. phaeochro-			
	F-105	–	+	mogenes	F-39	–	+
S. autotrophicus	F-88	–	+	A. prunicolor	F-2	++	+++
	F-118	–	+	S. puniceus	F-24	–	+
A. violascens	F-50	+	+++		F-32	–	–
	F-53	+	+++		F-58	–	–
	F-77	++	+++	Streptomyces sp.	F-41	–	++
	F-103	++	++		F-42	–	++
S. zaomyceticus	F-93	–	+		F-43	–	+
Streptomyces sp.	F-76	–	+		F-56	+ 5mm+	–
	F-81	+	+		F-62	+	+++
	F-86	++	+++		F-97	+	+
	F-6	–	–		F-137	–	–
	F-7	–	–		F-140	–	–
	F-35	–	+		F-147	–	–
					F-152	+	+++
					F-156	–	++

A = Actinomyces; S = Streptomyces; + Thickness of the colour ring.

28 °C. After 24, 48 and 72 hours the quantity of quinoid oxidation induced by phenol oxydases of colour reaction character, was evaluated on the phenol compounds tested, and compared to the control plates.

Results and discussion

Results of the phenol oxydase tests are demonstrated in Tables 1, 2 and 3, indicating the phenol oxydase reaction and its degree. Data obtained show that Streptomyces strains originating from the undisturbed topsoil and from the two variants of the recultivated dump o-polyphenol oxydases, first of all. Such type of endocellularly formed enzyme produces black-coloured o-quinone components in the presence of catechol. The typical colouration is localized only on the surface of the agar plate disc cut out from Streptomyces culture.

In some cases the presence of exo-cellulary p-phenol oxydase was also proven. Positive phenol oxydase testing showed dark brown colouration on the agar plate containing quinol. In case of high enzyme concentration the colouration significantly runs over the edge of the agar plate disc, forming a typical brown-coloured ring.

Tyrosinase and cresolase activity was registered in only one species. This strain belongs to the Streptomyces flora of the Pannonian clay dump. Strikingly, the control species representing the Streptomycete communities of Pannonian clay dump showed the most active phenol oxydase synthesis.

Table 2

The phenol oxidase reaction of the Streptomyces strains originating from the control variant of the Pannonian clay dump

Designation of investigated strains		Quinol	Catechol	Designation of investigated strains		Quinol	Catechol
S. actuosus	III/1-2	-	+	S. gannmycicus	III/1-14	-	+
	III/1-15	+	+++	S. massasporeus	III/1-33	-	+
	III/1-24	-	+		III/1-68	-	++
	III/1-28	+	++	S. parvus	III/1-36	-	+
S. alni	III/1-49	+	-		III/1-47	-	+
A. aurantio-griseus	III/1-37	++	++	S. peruviensis	III/1-12	++	++
S. capreolus	III/1-50	-	-	S. phaeochro-mogenes	III/1-7	++	+++
S. cellulosae	III/1-9	-	-		III/1-13	++	+++
	III/1-10	-	-		III/1-44	-	+
	III/1-73	-	-	S. saraceticus	III/1-19	+	++
A. cyaneo-fuscatus	III/1-52	+	+	p-Cresol; D-Tyrosine:		++	+++
S. diastatochro-mogenes	III/1-39	-	+	S. spadicis	III/1-38	-	+
	III/1-51	-	+		III/1-66	-	+
	III/1-67	-	+	S. violaceoruber	III/1-58	-	+
S. exfoliatus	III/1-5	-	+	Streptomyces sp.	III/1-17	-	++
	III/1-21	-	+		III/1-26	-	++
	III/1-48	-	+		III/1-29	+	+++
S. flavogriseus	III/1-43	-	-		III/1-35	-	+
	III/1-75	-	-		III/1-71	-	++
					III/1-72	+ 8mm[+]	-

[+]Thickness of the colour ring

Table 3

The phenol oxidase reaction of the Streptomycetes strains originating from the NPK + lignite variant of the Pannonian clay dump

Designation of investigated strains		Quinol	Catechol	Designation of investigated strains		Quinol	Catechol
S. violaceoruber	I/1-1	+	++	S. massasporeus	I/1-5	-	+
	I/1-35	+	+	S. olivaceus	I/1-84	-	+
	I/1-65	-	+	S. parvus	I/1-8	-	+
	I/1-103	-	+		I/1-61	-	+
	I/1-117	-	+		I/1-70	-	+
S. actuosus	I/1-49	+	++		I/1-90	-	+
S. alboniger	I/1-115	-	+	S. phaeochromogenes	I/1-43	++	+
S. capreolus	I/1-47	-	-	S. plicatus	I/1-78	-	+
S. flavogriseus	I/1-34	-	+		I/1-97	-	+
	I/1-41	-	-	A. prunicolor	I/1-114	-	-
S. flaveolus	I/1-27	-	+	S. somaliensis	I/1-125	-	-
	I/1-36	-	+	S. tetanusemus	I/1-24	-	-
A. fumanus	I/1-62	+	+	Streptomyces sp.	I/1-10	-	+
	I/1-81	+	+		I/1-40	-	+
S. griseolus	I/1-20	+6mm[+]	-		I/1-75	-	+
A. kurssanovii	I/1-32	-	++		I/1-76	-	+
	I/1-33	-	++		I/1-85	+	+
S. lincolnensis	I/1-99	++	++		I/1-110	-	-

[+] Thickness of the colour ring

Summary

Phenol oxydase enzyme synthesis of Streptomyces strains isolated and identified from the undisturbed topsoil of Gyöngyösvisonta /chernozem brown forest soil/ and from the controls and NPK + lignite-treated samples of a recultivation experiment set up with Pannonian clay dumps have been investigated.

Results show that the Streptomyces species originating from the undisturbed topsoil and the two variants of recultivated dumps form endocellulary o-polyphenol-oxydases, first of all. In some cases the presence of exocellularly p-phenol oxydase was also proven.

The control species representing the Streptomycete communities of clay dump showed the most active phenol oxydase synthesis. The only species showing positive tyrosinase and cresolase activity is also a member of this community.

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