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Regulation of siderophore production by iron Fe(III) in certain fungi and fluorescent pseudomonads

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Regulation of siderophore production in response to iron concentration in the medium was examined. Threshold concentration was recorded for twenty fungi and three rhizobacterial pseudomonads. Organisms showed difference in threshold values at which they stopped siderophore elaboration. In nine fungi (3 aspergilli, 1 penicillium, *N. crassa*, *F. dimerum* and 3 mucors) siderophore production was repressed at 3 μM Fe(III). Siderophore production was repressed at 27μM of Fe (III) in 3 aspergilli, 2 penicillia and 3 pseudomonads. Rest of the fungi had cut off values at 6,9,15,21μM of Fe(III) concentration.

Since reporting of microbial requirement of iron[1], much emphasis is laid on its role, and involvement in metabolic processes ranging from respiration to nucleic acid synthesis[2], except lactobacilli that utilize manganese and cobalt as biocatalysts in place of iron[3], all microorganisms do require iron. Its assimilation poses two problems for microbes—(a) they must overcome the insolubility of iron, and (b) they must regulate its uptake because of iron's potential for wreaking cellular metabolism. Despite the fact that iron is the fourth most abundant element in the earth's crust, it is unavailable for uptake as it occurs in the form of insoluble, stable polymers of ferric oxyhydroxide (geothite, hematite, etc.) at neutral to alkaline (*p*H4) condition of the soil in which the earth abounds. Hence, Fe(III) in an aerobic, aqueous environment is limited to an equilibrium concentration of $10^{-17}M$, a value far below that is required for the optimum growth of microbes (10^{-8}-$10^{-6}M$)[5] . In response, microorganisms rely on chelators, which they may or may not synthesize themselves to solubilize iron. These chelators have been termed siderophores[6] (Gr. iron-bearers) which are low molecular weight, ferric specific ligands, produced under iron-stress conditions. Thus, iron stress is the decisive factor affecting siderophore biosynthesis[7]. Mechanism of iron regulation of siderophore synthesis by iron in enteric bacteria is known where an iron-binding protein, *fur* protein functions as a repressor and ferrous ion as a corepressor [8]. However, the actual mechanism is still under investigation in fungi except in *Ustilago maydis*[9], where protein urbs 1 is involved in iron regulation. The objective of the present study was to examine the threshold value at which the ambient concentration represses siderophore production in fungi and bacteria (fluorescent pseudomonads).

Twenty fungi belonging to Zygomycotina (5, all Mucorales), Ascomycotina (7 aspergilli, 6 penicillia, *Neurospora crassa*) and Deuteromycotina (1, *Fusarium dimerum)* used in this study were supplied by the Head, Division of Plant Pathology, IARI, New Delhi. These fungi were maintained on potato dextrose agar (PDA) slants and stored at 4°C. The three fluorescent pseudomonads (P_1, P_2 and P_3) were from our own laboratory rhizosphere isolates. They were maintained on King's B medium (KB)[10] and stored at 4°C. All these fungi and fluorescent pseudomonads produced siderophores as evidenced by $FeCl_3$ test[11], Chrome azurol S (CAS) assay[12], CAS agar plate test[12] and the spectrophotometric assay characteristic of pyoverdine siderophore in fluorescent pseudomonads. All glasswares were treated with HCl (6*M*) to remove traces of iron. For fungi belonging to Ascomycotina and Deuteromycotina, Grimm–Allen[13] medium was used for siderophore production containing (per liter of distilled water) K_2SO_4, 1g; K_2HPO_4, 3g ; ammonium acetate, 3g; citric acid, 1g; sucrose, 20g; adjusted to *p*H 6.8 with ammonia. Siderophore production by fungi belonging to Mucorales, which failed to grow on Grimm – Allen medium was seen in Modified M9 medium[14], comprising (per liter of distilled water) glucose, 10g; Na_2HPO_4, 7g; KH_2PO_4, 3g; NaCl, 0.5g; NH_4Cl, 1g; $MgSO_4$. $7H_2O$, 0.25g; $CaCl_2$, 0.015g; adjusted to *p*H 7.2 with NaOH. For siderophore production by fluore-

Table 1 —Threshold concentration of $FeCl_3$ for siderophore production/repression by fungi and bacteria

	Test organism $FeCl_3$ concentration (μM)									
	1.5	3	6	9	12	15	18	21	24	27
					Fungi					
Aspergillus fumigatus	+ 0.09	—	—	—	—	—	—	—	—	—
A. melleus	+ 0.36	+ 0.29	+ 0.15	+ 0.14	+ 0.08	+ 0.08	+ 0.08	+ 0.07	+ 0.03	—
A.niger	+ 0.15	+ 0.13	+ 0.11	+ 0.10	+ 0.07	+ 0.06	+ 0.04	+ 0.03	+ 0.02	—
A.duricaulis	+ 0.50	+ 0.46	+ 0.40	+ 0.38	+ 0.28	+ 0.27	+ 0.27	+ 0.21	+ 0.12	—
A.versicolor	+ 0.08	—	—	—	—	—	—	—	—	—
A.deflectus	+ 0.08	—	—	—	—	—	—	—	—	—
A.ochraceus	+ 0.27	+ 0.24	+ 0.14	—	—	—	—	—	—	—
Penicillium chrysogenum	+ 0.30	+ 0.21	+ 0.16	+ 0.13	+ 0.10	+ 0.10	+ 0.99	+ 0.50	+ 0.40	—
P.camemberti	+ 0.21	+ 0.16	+ 0.15	+ 0.14	—	—	—	—	—	—
P.rugulosum	+ 0.25	+ 0.19	+ 0.16	+ 0.10	+ 0.06	+ 0.03	+ 0.02	+ 0.02	—	—
P.griseofulvum	+ 0.45	+ 0.40	+ 0.32	+ 0.22	+ 0.11	+ 0.05	—	—	—	—
P.notatum	+ 0.43	+ 0.32	+ 0.29	+ 0.20	+ 0.19	+ 0.15	+ 0.12	+ 0.11	+ 0.02	—
P.citrinum	+ 0.07	—	—	—	—	—	—	—	—	—
Neurospora crassa	+ 0.06	—	—	—	—	—	—	—	—	—
Fusarium dimerum	+ 0.09	—	—	—	—	—	—	—	—	—
Rhizopus rhizopodiformis	+ 0.30	+ 0.12	+ 0.09	+ 0.08	+ 0.08	+ 0.07	+ 0.06	+ 0.05	—	—
R. oryzae	+ 0.05	—	—	—	—	—	—	—	—	—
Cunninghamella elegans	+ 0.08	—	—	—	—	—	—	—	—	—
C.echinulata	+ 0.36	+ 0.17	+ 0.07	+ 0.02	—	—	—	—	—	—
Mucor mucedo	+ 0.07	—	—	—	—	—	—	—	—	—
Pseudomonas isolates					Bacteria					
P_1	+ 0.40	+ 0.35	+ 0.31	+ 0.29	+ 0.24	+ 0.22	+ 0.21	+ 0.19	+ 0.11	—
P_2	+ 0.50	+ 0.43	+ 0.39	+ 0.37	+ 0.35	+ 0.32	+ 0.22	+ 0.12	+ 0.11	—
P_3	+ 0.43	+ 0.32	+ 0.29	+ 0.28	+ 0.25	+ 0.19	+ 0.17	+ 0.11	+ 0.09	—

(+) – induction ; and (-) repression. The values indicate CAS activity at OD 630 nm.

scent pseudomonads, 24hr old culture was used to inoculate succinate medium[15](succinic acid, 4g; $(NH)_2SO_4$, 1g; KH_2PO_4, 0.1g; $MgSO_4$. $7H_2O$, 0.2g; adjusted to *p*H 7.2). These media were rendered iron—free by treating with 8—hydroxyquinoline dissolved in chloroform[2] and were supplemented with 0.25 to 27 μ*M* Fe (III). The cell-free supernatants of fungal and bacterial cultures were assayed for presence of siderophore by measuring CAS activity (OD at 630 nm[16]). Regulation of siderophore production was determined by ambient threshold concentration of available Fe (III) above which siderophore production was repressed. Opposite would happen, if the available Fe (III) is below the threshold value, i.e. siderophore production would be induced.

Results using 15 days old fungal and 48hr old bacterial cultures grown at 30°C, are shown in Table 1. It is evident that the fungi (Table1) showed much difference in the values at which siderophore production was repressed. Least concentration at which siderophore production was repressed was 3 μ*M* of Fe(III), as noted for nine fungi (*A. fumigatus, A. versicolor, A. deflectus, P. citrinum, N. crassa, F. dimerum, R. oryzae, C. elegans, M. mucedo*). However, five fungi (*A. melleus, A. niger, A. duricaulis, P. notatum, P. chrysogenum*) continued producing siderophores upto 24 μ*M* of Fe(III) concentration. At higher concentration of Fe (III), siderophore production was repressed. In case of *P. rugulosum* and *R. rhizopodiformis,* siderophore production was repressed at 24 μ*M* Fe(III), while in *P. camemberti* and *C. echinulata,* 12 μ*M* was the threshold concentration. *A. ochraceus* and *P. griseofulvum* were the lone examples where siderophore synthesis was repressed at concentration 9 and 18μ*M* Fe(III). Unlike fungi, the three *Pseudomonas* isolates (Table 1) showed a uniform behaviour as their siderophore production was repressed only at 27 μ*M*. Siderophore production as CAS activity gradually decreased with increasing iron concentration.

Non-production of siderophores by 9 fungi at the concentration above 3 μ*M* of Fe(III), suggested that they were iron-efficient as compared to others. All the fungi required siderophores for mobilization and uptake of iron. Based on the threshold values of siderophore repression, test fungi could be classified into six groups [i.e., fungi showed threshold value at 3, 6, 9, 15, 21 and 27 μ*M* of Fe (III)]. It has been reported earlier that 5μ*M* of Fe (III) is sufficient for the growth of most fungi[8]. Threshold value for siderophore repression in *Ustilago maydis* is reported to be 10 μ*M* of Fe(III)[17]. In fluorescent pseudomonads, siderophore production was repressed above 27 μ*M* of Fe(III). Differences between fungi and bacteria could be due to diversity among the test fungi comprising aspergilli, penicillia, mucors etc. belonging to various ecological niches, while the three pseudomonads belongs to a homogenous cluster – all fluorescent *Pseudomonas* and one specialized ecological niche – (the root surface).

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