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Study on Siderophore Production by Food Contaminant *Pseudomonas* spp

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The present investigation was undertaken to study siderophore production by *Pseudomonas* spp. isolated from spoiled food. Maximum siderophore productions i.e. 30mg/l were produced by Pseudomonas spp. of spoiled meat. Optimization study of cultural condition showed that maximum siderophore production was found at pH 7.0, temperature 30°C and iron concentration 10μM.

Key words: Siderophore Production, Food Contaminants, *Pseudomonas* spp.

Siderophore is an iron chelating compound secreted by microorganism having affinity for ferric ions (Lankford, 1973). It is synthesized under iron deficient condition by variety of microorganism such as bacteria and fungi. Many gram negative bacteria are known to produce one or more siderophore and the components for their transport. Such system has been well studied in *E. coli* (Bagg & Neilands, 1987, Wandersman & Delepelaire, 2004). They are classified based on, their chelating group specific for ferric iron. There are two main siderophore classes, the catechol-type and the hydroxamate-type (Wandersman & Delepelaire, 2004).

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Recently siderophore has gained application in biotechnology, agriculture and medicine. For example it is used in haemochromatosis disorder caused by deposition of iron in body. Administration of desferrioxamine which is form of siderophore lowers the level of aluminum in the body and relives the symptoms of the disease (Ackrill *et al*., 1980, Arze *et al*., 1981, Pogglistsch *et al*., 1981). Desferrioxamine has also been used to remove vanadium in rat. Desferal reduced the vanadium content in kidney by 20%in lung by 25% and in liver by 26% when administered at 100 m $molKg^{-1}$ following a dose 5 µ $molKg^{-1}$ of Na^{48} VO^{3}. Both urinary and faecal excretion increased at this dose (Hansen *et al*., 1982). Similarly siderophore from *Klebsiella pneumonia* has been used as an antimalarial agent (Gysin *et al*., 1991) and in cosmetics as deodorants (Johnsan *et al*., 2003). In agriculture inoculation of soil with *Pseudomonas putida* which produces pseudobactin increases growth and yield of various plants (Kloepper *et al*., 1980). To fulfill the demand of siderophore for above application it is

important to study a bacterial strain which having high siderophore producing capacity.

The present investigation was undertaken to assess the potential of *Pseudomonas spp*. isolated from contaminated food for siderophore production.

MATERIAL AND METHODS

The five *Pseudomonas* strains were isolated from different spoiled food such as meat, egg, fish, milk and cheese on citramide agar by serial dilution. The plates were incubated at 30°C for 24h. Colonies were identified as described by Gupte, (2002).

Siderophore production

Siderophore production by the *Pseudomonas* spp. was tested by chromo azural S (CAS) assay (Schwyan and Neilands, 1987). Siderophore production was also checked by the top layer method. The strains were spread over citramide agar and incubated for 48h at 30°C.After incubation a thin layer of CAS reagent in 0.7% agar was spread on the bacterial growth and plates were again incubated for 24h at 30°C formation of yellow orange zone around the colonies indicates siderophore production.

Siderophore assays

To determine the type of siderophore, isolated *Pseudomonas* strains were grown on Succinate medium (Mayer and Abdullah, 1978). Containing (gm/l) Succinic acid 4, K_2HPO_4 6, KH_2PO_4 3, $(NH_4)_2SO_4$ 1, $MgSO_4$ 0.2, and pH, 7.0. These strains were inoculated and incubated on rotatory shaking incubator for 36h at 28°C. A 5ml culture supernatant was harvested by centrifuging the culture at 10,000 rpm in cooling centrifuge at 4°C for 10min and supernatant were used. The presence of catechol type siderophore was tested according to Arnow method (Aronw, 1937) by taking 2,3 dihydroxybenzoyl acid as the standard and the presence of hydroxamate siderophore in the supernatant was checked according to Casky method (Gillan *et al*., 1981).

Effect of incubation period on siderophore production

The *Pseudomonas* strain was separately grown in succinate medium with constant shaking of 120 rpm at 30°C for 48h. Samples were withdrawn after every 6h of intervals and were subjected for siderophore estimation.

Effect of iron concentration on Siderophore production

To determine the effect of iron concentration the *Pseudomonas* strain were grown in succinate medium containing $Fecl_3$ in increasing amount i.e. 1-100mM. The flask was incubated for 24-30h at 28°C with constant shaking at 120 rpm on rotator shaking incubator.

Effect of pH on siderophore production

To evaluate siderophore production the pH of succinate medium was adjusted at 5, 6,7,8,9 and 10pH before inoculation with 1 N HCL or 1N NaoH by keeping all other condition at their optimum level.

RESULTS AND DISCUSSION

The present study revealed that all isolate and identified strain of *Pseudomonas* showed siderophore production on chromo azurol S agar by developing yellow to orange coloured zone around the colonies on agar plate similar finding were reported by Sayyed *et al* (2005). To determine

Table 1. Hydroxamate and Catecholate type of siderophore production by different *Pseudomonas* strains

S. No	Bacterial Strain	Siderophore production	
		Hydroxamatemg/l	Catecholatemg/l
1	*Pseudomonas spp. of cheese*	20.0	05.0
2	*Pseudomonas spp. of milk*	20.0	08.0
3	*Pseudomonas spp of fish*	28.0	05.0
4	*Pseudomonas spp. of egg*	25.0	07.0
5	*Pseudomonas spp. of meat*	30.0	08.0

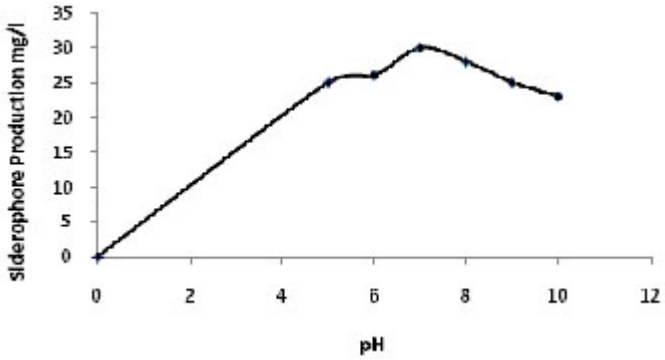

Fig. 1. Effect of pH on Siderophore production

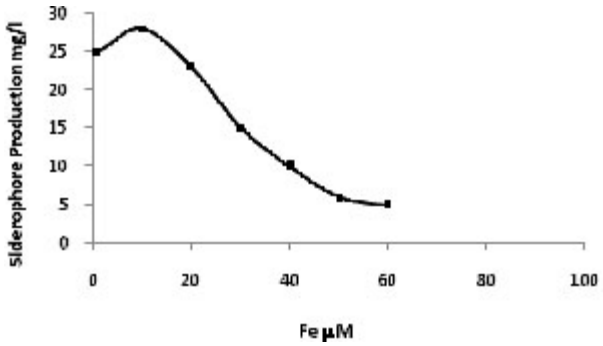

Fig. 2. Effect of iron concentration on Siderophore production

the type of siderophore the isolated strain of *Pseudomonas* was grown on succinate medium and supernatant were tested by Casky and Arnow assay. The result indicated that all isolate are positive in production of hydroxamate and chatcholate type of siderophore. This agrees with the study carried out by Kloepper *et al* (1980). But the amount varied in *Pseudomonas* strain isolated from meat shown in (Table 1). In meat product iron is tightly bound to protein such as hemoglobin, transferring, lactoferrin and ferritin. The strict homeostasis of iron leads to a free concentration of about 10^{-24}molL^{-1} (Raymond *et al*., 2003). These indicate that iron is important factor for production of siderophore.

As *Pseudomonas* strain isolated from meat showed maximum siderophore production further evaluation of the siderophore production studied continued with same strain. A maximum siderophore production was found on 24-36 h of incubation and pH 7.0 shown in Fig 1. This is due to bacteria grow better and iron is present in soluble form at neutral pH and therefore, is not available to the bacteria (Sayyed, *et al*., 2005). Siderophore are iron specific compounds which are secreted under low iron stress conditions. The optimal iron concentration for maximum siderophore production was studied at 10mM in succinate medium, while production of siderophore repressed when iron concentration is increased (Fig 2). Similar result was obtained by Raaska *et al*., (1993). Who examined detection of siderophore in growing culture of *Pseudomonas* spp.

CONCLUSION

The above results indicate that *Pseudomonas* strain of spoiled meat shows maximum siderophore production. And the production can increase by optimization of cultural condition, it was found that maximum siderophore production was achieved at optimum pH 7.0; temperature 30°C and iron concentration was 10µM. Hence the strain of *Pseudomonas* spp. can be employed for large scale siderophore production.

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