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PrfA protein of *L. monocytogenes* functions as a main factor required for the differential expression of bacterial virulence gene products

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ABSTRACT

PrfA protein is a transcriptional activator essential for *L. monocytogenes* pathogenicity and virulence upon invasion of mammalian cells. Its functional activity is better accomplished when it interacts and forms complexes with the prf-A dependant gene promoters like *prfA*P1/2, *plcA*, hly and others. PrfA is thought to acquire special functions required for differential expression of bacterial virulence gene products. This could occur when *L. monocytogenes* is primed by environmental cues which might enable the pathogen adapt to the survival challenges and succeed in virulence induction in the host. In addition, emerging mutant strains of PrfA have also turned out to exhibit differential activity in DNA binding and transcription activities. This could indicate that PrfA protein of *L. monocytogenes* has evolved in its associations with the gene promoters that lead to diverse outcomes related to the bacterial gene virulence. The differential role of PrfA in promoting virulent gene expression has implications in studying the functional outcomes of PrfA-novel gene promoter complexes in a given pathogenic environment.

KEYWORDS

PrfA protein;
L. monocytogenes;
Promoters;
Differential expression;
Virulence.

INTRODUCTION

PrfA is a transcriptional activator protein of facultative bacterium, *Listeria monocytogenes*. This microbe is an opportunistic intracellular pathogen mainly responsible for human foodborne infections worldwide[1]. The pathogen especially causes Listerosis in humans upon invasion and is found in wild and domestic animals, water and soil[2]. PrfA, is a27 kDa DNA binding protein and belongs to Crp/Fnr family of transcriptional activators[3]. It is very specific in recognizing PrfA-dependent promoters that contain a 14-bp palindrome, PrfA box[4]. The PrfA protein is homologous to *Escherichia coli* cyclic AMP (cAMP) receptor protein (Crp) in structure and function[5].

PrfA gene is considered as the main contributor of *L. monocytogenes* pathogenesis Its depletion from the bacterial strains was also reported to result in non- virulence in mouse models of infection[6,7].

PrfA gene which encodes PrfA protein is controlled by three unique promoters namely, *prfA*P1, *prfA*P2 and *plcA*[6,8,9]. PrfA is thought to mainly interact with plcA promoter and undergoes a positive self –regulated expression. This results in *prfA–plcA* transcript

formation and an increased PrfA synthesis that becomes pivotal for the bacterial intracellular spread and virulence[6,9].

PrfA serves as a virulence regulator as it coordinates and differentially controls the cascade of events in the infection process. These are eukaryotic cell internalization, phagosome lysis, host cytoplasmic multiplication and motility, intracellular distribution and final double-membrane vacuole lysis during neighboring cell entry[10].

Interestingly, this diverse regulatory action of PrfA has not been given adequate research focus in the recent years. Therefore, this has drawn our attention to carry out a review study on a concept that highlights PrfA led differential expression of bacterial virulence gene products.

It was reported that PrfA gene undergoes sequence variation at its binding sites which increase its capability to interact differentially with the promoters[6,11].

Researchers have tested the differential virulence activity of PrfA by choosing a heterologous host *Bacillus subtilis*[5]. PrfA was found to exert its regulating effect by activating the expression of hly, plcA, mpl and actA promoter genes. Here,for the activation hly-plcA promoter, a low PrfA concentration and for activation of actA and mpl promoters, a high PrfA concentration proved to be essential. This indicated an inverse correlation between PrfA amounts and promoter activations[5].

The differential Prf-a activation was attributed to single base substitutions of the PrfA targeted promoter sites which could alter or lessen the efficacy of PrfA led gene expression. Especially, transcription activation at the plcA promoter occurs an autocatalytic circuit mode. As such, prfA-encoding mRNAs accumulate at increased proportion leading to differential gene expression system. Ultimately, L. monocytogenes infection process gets accelerated and phagolysosomal lysis factors undergo earlier expression following activation of virulence genes in sequence[5].

NEED OF ADDITIONAL REGULATORY COMPONENTS

PrfA differential activity in *L. monocytogenes* relies on additional regulatory components. This has become apparent when PrfA led *inl-A* promoter expression was compared between the heterlogous *B.subtilis* and *L. monocytogenes*[5]. Here, immunoblot findings revealed a presence of fivefold lower levels of inl- AB in *B.subtilis* than *L. monocytogenes*. In *L. monocytogenes*, PrfA interaction with the putative sites in the inlA promoter region could require higher levels of PrfA that are absent in *B. subtilis* system. Moreover, PrfA led inlA expression was also reported to be partial due to the necessity of additional regulatory components when a DprfA mutant strain BUG802 was found with significant inlAB locus transcription[5]. PrfA was even found to be in need of higher concentration of additional factors when intergenic plcA-prfA region was fused with lac- z system specific to *B.subtilis*[12]. This is because PrfA was unable to sufficiently activate transcription of the inlA-lacZ fusion in *B. subtilis*[12].

The other reasons are that monocistronic prfA message levels do not get altered by environmental stimuli[13]. Actually, in Listeriae members, transcription of virulence determining genes and/ or promoters like hly and plcA genes are inhibited in the presence of low temperature[14] and 5 to 10 mM cellobiose growth conditions[15]. As PrfA was found resistant to environmental cues, it suggested that PrfA dependant gene regulation and differential virulence property of genes could have been evolved with special mechanisms in *L. monocytogenes*.

However, further studies are largely needed to dissect the association between specific involvement of regulatory components and PrfA differential functional activity.

Next, PrfA dependant differential gene regulation could depend on functional activity of PrfA mutant strains.

Prf-A MUTANT STRAINS

Briefly, mutant forms its products encoded by *L. monocytogenes* were described to be feeble in generating virulence once they get attenuated following cytosol entry[16]. But scientists were able to isolate *L. monocytogenes* strains, with mutations within *prfA*, termed as *prfA** alleles which could undergo constitutive PrfA activation[17-22]. *PrfA** strains have increased host cell invasion capability, instant escape from phagosome and also virulence inducing characteristics *in vivo* in mice[17,18]. These mutants are similar to wild type strains in PrfA-dependent gene expression found in

Review

bacteria during intracellular growth[18,23-25].

In connection with this when prfA mutation studies were conducted, it was observed that *certain PrfA** mutant strains were able to exhibit competitive advantage over wild type *PrfA* strains through an increased utilization of glycerol but not glucose as the main carbon source in culture media[26]. When assessed for five cycles of culture dilution and outgrowth, wild type *L. monocytogenes strains were found to possess* low levels of PrfA-dependent gene expression than the isogenic *prfA** mutants in terms of glycerol utilization and gene expression.

PrfA* mutants, thus, are made to undergo a metabolic shift in utilizing carbon sources that promotes bacterial growth in the cytosol and successful replication in the eukaryotic cells[26].

This could indicate that depending on the environmental stimuli Prf-A protein could modulate its activity within a host by an orchestrated switching from the wild type to a mutant type. So, the virulence in bacterial genes observed in *L.monocytogenes* may be due to differential activity PrfA mutant forms.

Vega et al.(2004) described PrfA* mutations such as Leu-140Phe and Ile-45Ser that cause PrfA regulon constitutive overexpression. These mutations exhibited differential target DNA binding property that correlated with differences in transcriptional activity. Especially, the affinity of PrfA for the PrfA-dependent promoters, PplcA and PactA was revealed to be high and low, respectively. In addition, PplcA was reported to possess greater affinity for RNA polymerase (RNAP) than PactA.

Thus, PrfA activity for the differential virulence gene regulation could be influenced by relative affinity of PrfA-dependent promoters for PrfA and RNA polymerase (RNAP) initiated transcription[27]. Interestingly, PrfA protein functional aspects are thought to be altered differently by mutations[18]. Scientists have identified mutations like PrfA G155S,PrfA E77K[18], *prfA* Y63C/Y154C[28,29], and prfA P219S[23]. This implies tthat mutation studies may have good future implications for further understanding the differential PrfA gene activity and its impact on virulence gene expression. Most importantly, problem s like poor bacterial resistance could be studied and solution could be sought through novel approaches that target the PrfA protein functional activity.

CONCLUSION

In conclusion, PrfA protein essential for Listeriosis appears to rely on a given environmental stimuli. This could trigger a necessity of factors like additional regulatory components, metabolic shifts, mutant driven activity for differential regulation of bacterial virulence gene products. Further studies are suggestive for strengthening this concept.

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Review

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