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Regulation of *Streptomyces* Chitinases by Two-Component Signal Transduction Systems and their Post Translational Modifications: A Review

Amrathlal Rabbind Singh*

Department of Genetic Engineering, School of Biotechnology,
Madurai Kamaraj University Madurai - 625 021 India.

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This article reviews the developments related to *Streptomyces* chitinases regulation and their post translational modifications. Chitinases are enzymes which cleave chitin, a polymer of N-acetylglucosamine to its monomer. Bacteria produce chitinases to fulfil their nutritional needs since by-products of chitin degradation can serve as a source of carbon and nitrogen. Chitinolytic bacteria such as *Streptomyces* produce multiple chitinases which act synergistically to degrade crystalline form of chitin. *Streptomyces* are one of the major producers of chitinases in the soil. Every *Streptomyces* genome sequenced till date has multiple genes for chitinases. The chitinases resulting from proteolytic cleavage have different specific activities and binding efficiency. Both of the above mentioned factors contribute to complexity of the chitinolytic system. Two component systems (TCS) are the predominant signal transduction system in bacteria that regulate a wide variety of behaviours as well as fundamental processes such as metabolism and motility. Bacteria generally use two-component signal transduction pathways to couple environmental stimuli to adaptive responses. Apart from the generalized behaviours they also regulate specialised processes such as development and virulence. Thus this review focuses on the two component systems of *Streptomyces*, their mechanism of action, regulation of chitinases by TCS and post-translational modifications.

Keywords: *Streptomyces*, Chitinase, Two-component systems, Glycosylation, Proteolytic cleavage.

Chitinases are widely distributed in various organisms ranging from bacteria [1] to humans [2]. These are enzymes that catalyze the hydrolysis of chitin, which is a β-1, 4 linked homopolymer of N-acetyl-D-glucosamine. Chitin is the second abundant polysaccharide found in nature, with the most abundant being cellulose [3, 4]. *Streptomyces* are the main decomposers of chitin present in soil [5] since they can use chitin as a source of carbon and nitrogen, thereby playing a significant role in the turnover of chitin [6]. Chitin was once considered a waste product, however, reports on applications of chitin and chitin derived products have emerged and so it attracts a special interest as a reusable material [7].

Chitinolysis is performed by three separate enzymes namely endochitinases which produce multimers of N-acetlyglucosamine (NAG), exochitinases which produce low molecular weight chitobiose that are subsequently hydrolyzed to NAG by chitobiases [8]. Multiple chitinase encoding genes are distributed at different locations on the chromosomes of *S. coeliocolor* [9], *S. avermitilis* [10] and *S. griseus* [11]. The location of various chitinase genes of three *Streptomyces* species is shown in table 1 A, B and C. Number of chitinase genes is quite high in *S. coelicolor* [9] in comparison to other chitinase producing bacteria such as *Bacillus circulans* [12] and *Serratia marcescens* [13].

* To whom all correspondence should be addressed.
Current address: Centre for Genomics,
Jiwaji University, Gwalior - 475 011, India.
E-mail: rabbind@gmail.com

Streptomyces chitinases display a modular structure with domains organized in following order from the N-terminus: signal peptide, substrate-binding domain, fibronectin-type III like domain and a catalytic domain [14]. A signal peptide for chitinase secretion is usually present along with the catalytic domain. The catalytic domain is necessary for hydrolysis of β-1, 4 glycosidic bonds linking the N-acetyl glucosamine subunits of chitin [15]. Chitinases are not the only enzymes having accessory domains in addition to catalytic domains, but several carbohydrases such as amylases, cellulases, xylanases and pectinases also possess additional domains [14]. The importance of chitin binding domains has been demonstrated only in a few bacterial chitinases [16, 17].

Chitinases undergoing post-translational modifications have been reported from *Streptomyces* and other bacteria [12, 18]. The most common post-translational modification observed in chitinases of *Streptomyces* is proteolytic processing. Proteolytic cleavage contributes to multiplicity of chitinases to a larger extent in *Streptomyces* [19] and *S. marcescens* [20]. Glycosylation, a complex co- or post-translational modification has also been identified in the proteins of archea and bacteria. Among *Streptomyces*, glycosylated chitinases have been reported to occur in *S. olivaceoviridis* and *S. griseus* [21, 22]. Apart from *Streptomyces* and *Cellulomonas*, glycosylated chitinases have also been identified in plants [23] and animals [24]. The role of proteolytic processing of chitinases is known but a clear understanding with respect to the significance of glycosylation in chitinases in bacteria is still lacking. However, the most speculated role for glycosylation is that it protects the proteins from proteases. This property has been demonstrated for proteins such as cellulase of *Cellulomonas fimi* [25] and xylanase of *S. lividans* [26] but not in the case of chitinases.

Chitin and its importance

Chitin is an essential structural component of the fungal cell wall and is also present in the exoskeleton of arthropods and the micro-filarial sheath of nematodes. Chitin acts as a protective layer against the harsh conditions that may be endured by the pathogen or arthropod. It is also the second most abundant glycol-polymer on earth, with an estimated 10^{10} tonnes of chitin produced each year next only to cellulose [27]. Chitin is composed largely of alternating by β-(1-4)-glycosidic bonds. Crystalline chitin occurs in three forms α-, β-, γ- chitin. The variation lies in the degree of hydration, size of unit cell and number of chitin chains per unit cell [28, 29]. α-chitin the most abundant form of chitin is a component of the fungal cell wall and arthropod exoskeleton, has very high tensile strength [30, 31]. b-chitin occurs less frequently in nature [30]. Chitin is also a component of spores produced by certain *Streptomyces* [31]. It is structurally identical to cellulose except that chitin has acetamide groups at the C-2 position. Although both cellulose and chitin have β- (1-4)-glycosidic bonds but they differ in properties. Chitin is hydrophobic whereas cellulose is hydrophilic [32]. Chitin contains 5-8% nitrogen mostly in the form of primary aliphatic amino groups [32]. Chitin derived products have found immense application in field of medicine. Chitin and its derivatives have been used in wound healing and tissue regeneration [32, 33], antioxidant properties have been demonstrated and can also be used as carriers in drug delivery. Use of chitin and chitosans as immunoadjuvants and non-allergic carriers of drugs has also been shown [34]. Chitin and its by-products have shown anti-microbial activity against *P. aeruginosa* besides applications in agriculture, detoxification and biotechnology.

Multiplicity of chitinases in *Streptomyces*

Most chitinase producing organisms produce multiple forms of chitinases. The isoforms of chitinases could result from products of multiple genes, post-translational proteolytic processing. Multiple chitinase genes have been identified in *Serratia marcescens* [13], *Aeromonas sp.* [7], *P. aeruginosa* [35], *B. circulans* [12] and *Streptomyces sps* [14, 36, 37]. Synergy between multiple chitinases is assumed to be important for effective chitin degradation [38]. Table 2 lists the *Streptomyces* species producing multiple chitinases. *Alteromonas* is a proficient degrader of chitin in the marine environments. Its capability is achieved by the production of four chitinases. All the four chitinases have varied substrate preferences and are induced in the presence of chitin. However, there was a variation in the level of transcripts relatively [38]. The results of Orikoshi *et al.,* (2005) indicated that ChiA plays a central role in chitin degradation process. Unlike *Alteromonas*, *Pseudoalteromonas* is also a marine bacterium that produces multiple

chitinases showing varied preferences to substrates [39]. ChiC of *Pseudoalteromonas* could be expressed in *E. coli* from its own promoter [39]. *S. coelicolor* too has genes for multiple chitinases. It produces both family 18 and family 19 chitinases, they show diversity in the multiple domain structures and also in their sequences [15, 36].

Two-component signal transduction systems (TCS)

Streptomyces are soil bacteria and thus need to adapt to a wide range of environmental conditions. To accomplish this they need to monitor these conditions and respond to changes accordingly. In bacteria sensing and adapting is mediated by two-component signal transduction systems (TCS) [40, 41]. Two-component systems confer the ability in microorganisms to adapt to changes in the environment by modifying the expression levels of genes in response to various stimuli [42]. This adaptation can include secretion of degradative enzymes, motility, virulence and changes that result in the modification of cell wall. Two-component systems function as intracellular information processing pathways that link external stimuli to specific adaptive responses.

The prototypical two-component regulatory system is composed of a sensor kinase (SK) and a response regulator (RR) [43]. Both sensor kinases and response regulators are modular proteins containing highly conserved modules. Sensor kinase consists of a variable N-terminal input (sensing) domain connected to a conserved C-terminal domain. A typical response regulator consists of a conserved N-terminal receiver domain and a variable C-terminal output domain. Two-component systems sense the signal through a membrane localized sensor kinase which undergoes phosphorylation at a specific histidine residue upon recognition of an appropriate signal. ATP acts as the phosphate donor in the phosphorylation process. The phosphorylated histidine residue transphosphorylates an aspartate residue located in its cognate response regulator. A scheme depicting the various steps involved in sensing and responding process is shown in figure 1. The control of longevity of the cellular response must be long enough for an effective response [44] but timely termination is necessary for the cell to adjust its behaviour as conditions change. Hydrolysis of the phosphoryl group from the response regulator resets the system to respond to additional stimuli. The process of hydrolysis can be performed by autophosphatase activity of RR [45], or phosphatase activity of SK [46, 47] or it can also be mediated aspartate phosphatases [48, 49]. Through this process, the phosphate group is transferred to an aspartate of a response regulator and finally released as inorganic phosphate. Phosphorylated response regulator binds to regions upstream of genes they regulate, often leading to enhanced target expression or to a reduced expression in few cases. Complex TCS also exist in which a histidine phosphotransferase mediates the transfer of phosphate from sensor kinase to the response regulator. Functioning mechanism of a complex TCS in which a sensor kinase is located in the membrane and the phosphotransfer reaction is mediated by a histidine phosphotransferase is shown as a scheme in figure 1. Through the mechanism of phosphorylation and phosphotransfer, bacteria use TCS to translate external signals into changes in gene expression, facilitating responses to environmental stimuli.

Although auto dephosphorylation of response regulator has been reported [45], in many cases it occurs through catalysis by another protein. Some SKs possess phosphatase activity towards their response regulator. Since sensor kinase and response regulator communicate via transphosphorylation reaction, this reaction requires the formation of precise but transient complex between the phosphorylation domain of SK and phospho-acceptor domain of RR [50].

In *S. coelicolor*, the total number of nucleotides from TCS genes takes up approximately 2.34% of the whole chromosome (Fig. 2). Based on the previous conclusion that bacteria distributed in diverse ecological niches, tend to encode larger number of TCS than those living in limited or obligatory environments [51, 52]. The total number of TCS proteins encoded by a bacterial genome, together with other signalling proteins, can be used as a measure of the adaptive potential of the organism (i.e., the bacterial intelligence quotient or "IQ") [52]. The genome sequence of *S. coelicolor* identified 84 genes encoding sensor kinase like proteins of which 67 are located adjacent to response regulator genes [9, 53]. Except for a few proteins most of them still remain uncharacterised. The signals that activate the sensor kinases in these

systems, the molecular details of phosphorelay reaction and sites in target DNA to which activated response regulators bind are defined only for a few TCS.

Sensor kinases

Sensor kinases are also referred to as sensor histidine kinases. In two-component systems, they function as receptors for stimuli and as regulators that control the activity of downstream signalling components via phosphorylation. From an enzymology perspective, sensor kinases are interesting because many of them participate in three distinct but related reactions such as autophosphorylation, phosphotransfer and dephosphorylation. Sensor kinases are modular proteins with distinct structural domains playing different functional roles. Most of them have an amino terminal sensor domain which is stimulus specific and not highly conserved at the sequence level [54]. The sensor domain involved in stimulus perception spans the membrane. The sensing domain is connected to a conserved cytoplasmic domain which has dimerization domain and phosphate accepting histidine (DHp). The DHp domain is in turn connected to catalytic and ATP binding domain (CA domain). A number of sensor kinases from *Streptomyces* have been identified and their functioning mechanism studied. Table 3 lists the identified SKs of *Streptomyces* and the method used for their characterization.

Sensor kinases can be categorised into two major groups. One group comprises the SKs of classical two-component signalling systems in which the kinase domain is at the carboxyl terminus of the protein. The other group comprises of hybrid sensor kinases which also contain a response regulator domain along with the kinase domains. Sensor kinases function as dimeric proteins that undergo autophosphorylation on a conserved histidine residue in response to specific stimuli [55]. They consist of an ATP binding kinase domain and a motif H-box containing the histidine residue which gets phosphorylated. The kinase domain consists of three conserved motifs namely, N, F, and G boxes [56]. Modular nature of sensor kinases and response regulators is shown in figure 1.

Based on the organisation of H-box and kinase domains as well as on differences in the amino acid sequences, five types of SKs were identified. Type I SKs predominate in bacteria where as in archea type II SKs predominate. Type III SKs predominate in gram-positive bacteria. Type IV were a minor type found in bacteria. Chemosensory sensor kinases belonged to type V [57]. All bacterial genomes sequenced till date contain SKs with mycoplasma being the only exception [58]. The number of SKs seemed to increase as the genome size increases [59]. Generally free living bacteria possess larger genomes when compared to pathogenic bacteria. Thus, the number of SKs was more in free living bacteria in comparison to the pathogenic forms [57].

Response regulators

Most of the signal transduction systems in bacteria are based on the central phosphate transfer mechanism involving two-components, a sensor kinase and a response regulator [41]. A response regulator is a two domain protein having N-terminal conserved receiver domain and a variable effector domain at the C-terminal end [60]. Even though sequence conservation in response regulators exists it cannot be used as an indicator of functional similarity, for example DivK of *Caulobacter crescentus* and Spo0F of *Bacillus subtilis*, share 30% identity but have completely different functions. Similar situation exists even for response regulators such as OmpR and PhoB where they share 37% identity but have unrelated functions [61]. This leads to problems in assigning function to RRs based on sequence similarity. Response regulators primarily exist in two different conformations, i.e. the active/ inactive form. Phosphorylation of receiver domain generates the activated form of response regulator in most cases [62, 63]. Domain rearrangements on phosphorylation have been identified in response regulators by solving the crystal structures of phosphorylated and non-phosphorylated forms [64]. In prokaryotes, response regulators are the terminal component in pathways functioning as phosphorylation activated switches to effect the response [41, 65]. They have the capability to catalyze phosphotransfer from histidine to aspartate and can also catalyze their phosphorylation from small molecule phosphodonors independently of SK [66-68]. Effector domains in RRs vary in function and some RRs even lack the effector domain [69]. The effector domain can have varied functions such as DNA binding, RNA binding [70], enzyme activity [40, 71] and also protein-protein interaction domain [72]. Modular

nature of response regulators is shown in figure 1.

Orphan sensor kinases and response regulators

Classical two-component regulatory systems are naturally encoded in locus that includes both sensor kinase (SK) and response regulator (RR) genes [73]. The organization of SKs and RRs in a locus favors co-expression of the corresponding proteins and decreases the chances of cross-talk between non-cognate SKs and RRs. However, genomes of bacteria also encode for solitary SKs and RRs also referred to as orphan sensor kinases and response regulators. Even though there is hardly any bacterial genome which does not have orphan sensor kinases and response regulators, there is a considerable variation in the number of orphan SKs and RRs. Two out of 32 sensor kinases in *E. coli* genome are categorized as orphan SKs [74]. This number is quite less when compared to *Caulobacter crescentus* genome in which 57% TCS genes exist as orphans [75]. Interestingly of the 84 sensor kinase genes identified in the *S. coelicolor* genome 67 are located in pair with their cognate response regulator gene and 17 genes were identified as orphan [53]. This shows that 20% sensor kinases in *S. coelicolor* are orphan sensor kinases. This number is quite less in comparison to *C. crescentus*. Few orphan sensor kinases specifically phoshorylate/interact with a particular response regulator which has been identified by *in vitro* phosphotransfer experiments using recombinant proteins. Interestingly, though orphan sensor kinases and response regulators lack their prototypical partners they function in very unorthodox ways to modify gene expression. Orphan Sensor kinases sometimes regulate primary functions mediated by a non-orphan TCS. This was observed in the case of GacS and GacA TCS of *Pseudomonas aeruginosa* where in RetS and GacS reciprocally control the expression of virulence factors responsible for acute and chronic infections [76]. The orphan RetS protein binds to GacS protein thereby, inhibiting its ability to autophosphorylate using ATP and also stimulating the dephosphorylation of GacS~P [76]. Non-binding of RetS to PilS proved its specificity towards GacS [77].

Annotation of the 3.3 Mb genome sequence of *Lactobacillus plantarum* WCFS1 revealed the presence of 13 paired TCS genes, and one orphan SK and RR [78]. SKs and RRs that belong to a common two-component signalling pathway are often encoded by genes that are organized as a locus on the bacterial chromosome. TarC, response regulator, shares no apparent association with a cognate HK. This was indicated by RT-PCR analysis, which reveals a single monocistronic mRNA that is derived from a promoter located immediately upstream of *tarC*. While it is unclear how orphan response regulators like TarC modulate gene expression, one could envision their involvement as intermediaries of *in vivo* cross talk between otherwise independent signal transduction systems. In fact, a recent report supports phosphoryl transfer from a sensory kinase to a non-cognate response regulator in *E. coli* [79], and cross talk has been suggested in the regulation of *S. mutans* acid tolerance by Li and co-workers [80].

Signal integration in TCS

To respond to diverse environmental changes with greater sensitivity, information is also conveyed between TCSs to form a complex signal transduction network [81]. In a classical TCS sensor kinase autophosphorylates and transfers the phosphate to its cognate response regulator. The phosphorylation of response regulator triggers conformational changes, due to which it is able to perform its designated function. However, complex TCS do exist, in bacteria these systems are designated as phosphorelays. In a typical phosphorelay the SK autophosphorylates and transfers the phosphate to a stand-alone RR, the phosphorylated RR serves as a phosphate donor for a SK which in turn transphosphorylates a RR which eventually performs its designated function [82]. There also exist a group of proteins whose primary function is to connect two TCS and they most often serve to connect two independent two-component systems. The connector proteins employ variety of strategies to perform their roles, the most important being inhibiting autophosphorylation of SK [83], promoting dephosphorylation of RR [48], inhibiting dephosphorylation of RR [84], activating a sensor kinase [85], inhibiting recruitment of RNA polymerase [86, 87] and sometimes by sequestering proteins which in turn promote protein degradation [88]. The connector proteins display distinct quantitative and kinetic properties that determine the timing and intensity of the response output. The genes which are regulated at the transcriptional level

by connector proteins often display an increased mRNA induction when compared to directly regulated genes.

Mathematical modeling demonstrated that PhoP/PhoQ, PmrA/PmrB of *Salmonella enterica* which are connected by PmrD protein [85] showed increased level of mRNA induction [84, 89, 90]. The connector proteins are also known to expand the spectrum of signals that influence the activity of RR [81]. Interaction of connector proteins and their targets is a highly specific reaction such that the connector proteins do not interact even with their closest homologs as observed in the case of PmrD were it seldom interacts with YgiX RR a close homolog of PmrA [91, 92].

The interaction between aspartate phosphatases RapA, RapB, RapE and Spo0E proteins is also a highly specific reaction as

Table 1. Chitinase genes present in the genome of *S. avermitilis*[10], *S. griseus* [11] and *S. coelicolor* [9]

S. No.	Enzyme/gene	Location on the chromosome (base) Start	End	No. of amino acid residues
1.	Chitinase A	2132343	2134028	561
2.	Chitinase B	3177737	3179566	609
3.	Chitinase C precursor	3522979	3524802	607
4.	Chitinase A	4054020	4055759	579
5.	Endochitinase	6815383	6816435	350
6.	Chitinase	8252433	8253716	427

S. No.	Enzyme/gene	Location on the chromosome (base) Start	End	No. of amino acid residues
1.	Chitinase II	2177892	2179715	607
2.	Chitinase III	2559778	2561649	623
3.	Putative chitinase	2898151	2899548	465
4.	Chitinase I	2990179	2991903	574
5.	Chitinase C	3985104	3985988	294
6.	Putative chitinase	3986289	3987110	273
7.	Putative chitinase	7176841	7179198	785
8.	Putative chitinase	7195071	7196342	423
9.	Putative chitinase	7757174	7758571	465
10.	Putative chitinase	8039719	8040786	355

S. No.	Enzyme/gene	Location on the chromosome (base) Start	End	No. of amino acid residues
1.	Secreted chitinase	5023432	503076	244
2.	Chitinase	1524785	1526038	417
3.	Chitinase precursor	1539609	1541984	791
4.	Chitinase precursor	2701242	2702318	358
5.	Chitinase A precursor	5439963	5441678	571
6.	Chitinase C	5845252	5847081	609
7.	Secreted chitinase	6172804	6174636	610
8.	Chitinase (Sec. protein)	6524142	6526439	765
9.	Secreted chitinase	6593034	6594557	507
10.	Secreted chitinase	7003454	7004212	252
11.	Secreted chitinase	8030688	8031422	244
12.	Chitinase	8073282	8074172	296

demonstrated [93]. Even though target specificity is predominant among connector proteins, few connector proteins also display dual functions such as in the case of RapH which it promotes dephosphorylation of Spo0F~P and also inhibits the DNA binding activity of response regulator ComA. Thus RapH controls both competence as well as sporulation [94].

Sensor kinases which can activate multiple RRs also known to exist, thus can feed multiple signals into a particular pathway. This phenomenon has been studied in great detail in the chemotaxis TCS of *E. coli* where swimming behavior is modified in response to changes in the concentration of different substrates. CheW a membrane protein senses the stimuli and alters

Table 2. Streptomyces expressing multiple chitinases

S. no	*Streptomyces* spp.	Chitinase genes	References
1.	*S. lividans*	*chiA*	104
		chiB	14
		chiC	131
2.	*S. olivaceoviridis*	*chiO1*	127
		chi92	127
3.	*S. thermoviolaceus*	*chi40*	113, 132, 133
		chi30	
		chi35	
		chi25	
4.	*S. griseus*	*chiC*	14, 128, 134
		chiI	
		chiII	
		chiIII	
5.	*S. peucetius*	*chiC*	1
		chiA	
6.	*S. coelicolor*	*chiA*	15, 36
		chiB	
		chiC	
		chiD	
		chiE	
		chiF	
		chiG	
		chiH	

Table 3. Characterized sensor kinases from *Streptomyces*

SK	Organism	Approaches used to characterize	Function regulated	References
AbsA1	*S. coelicolor*	Genetic, Biochemical	Antibiotic production	135-137
SenS	*S. reticuli*	Genetic, Biochemical	Production of catalase-peroxidase	138, 139
ChiS	*S. coelicolor, S. thermoviolaceus*	Genetic	Chitinase	113, 114
EcrA1	*S. coelicolor*	Genetic	Antibioticproduction	140
VanS	*S. coelicolor*	Genetic, Biochemical	Resistance to vancomycin	141
PhoR	*S. lividans*	Genetic	Alkaline phosphatase and antibiotics	142
RapA1	*S. coelicolor*	Genomic, Proteomic	Antibiotic production	143
CseC	*S. coelicolor*	Genetic	Sigma factor	144
AfsQ1	*S. coelicolorS. lividans*	Genetic	Secondary metabolites	145

the phosphorylated state of CheA (sensor kinase) which results in the phosphorylation of CheB and CheY (response regulators) [95]. The phosphorylation state of either CheY or CheB dictates the swimming behavior which can be smooth or tumble. The change in swimming pattern is modulated by the interaction of RRs with flagellar motor protein. *E. coli* has five sensory receptors which are localized at the bacterial poles. These proteins form sensory complexes by teaming up with CheA and CheW thus enabling processing of multiple signals at one time [96]. Marine bacterium *Vibrio harveyi* responds to two types of auto-inducer (AI) molecules known as AI-1 and AI-2. AI-1 is produced specifically by *V. harveyi* and AI-2 is a product of metabolism from wide variety of bacteria. The response to the presence of AI-1 and AI-2 is mediated by LuxN and LuxQ which acts via a periplasmic protein LuxP. All these sensors converge in phosphotransfer domain containing protein LuxU which in turn phosphorylates the LuxO a response regulator [97]. Phosphorylated RR activates a repressor which turns off the genes for bioluminescence. The convergence of signal originating from two sensors onto a single RR help *V. harveyi* in responding to its own cell density as well as from other bacteria [96].

TCS regulating extracellular enzymes

Bacteria possess multiple signal transduction pathways to adapt to changes in the environment. The changes in the environment which bacteria sense and respond can be of either biotic or abiotic in nature. The capacity to utilize variety of nutrients is highly developed in actinomycetes. *Bacillus subtilis* produces a variety of degradative enzymes which enable the bacterium

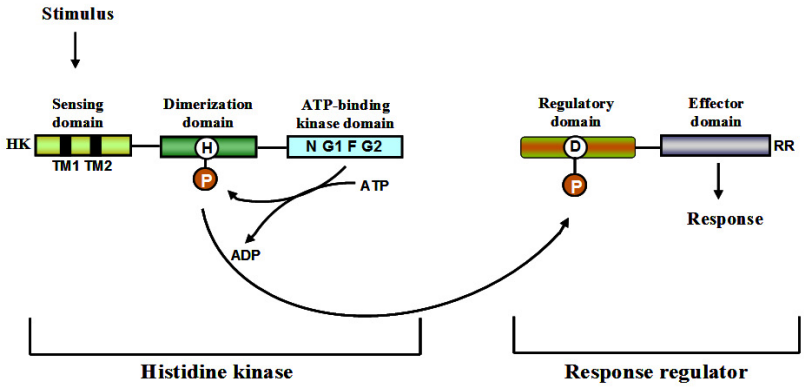

Fig. 1. Schematic representation of the conserved domains present in sensor kinases and response regulators [146]

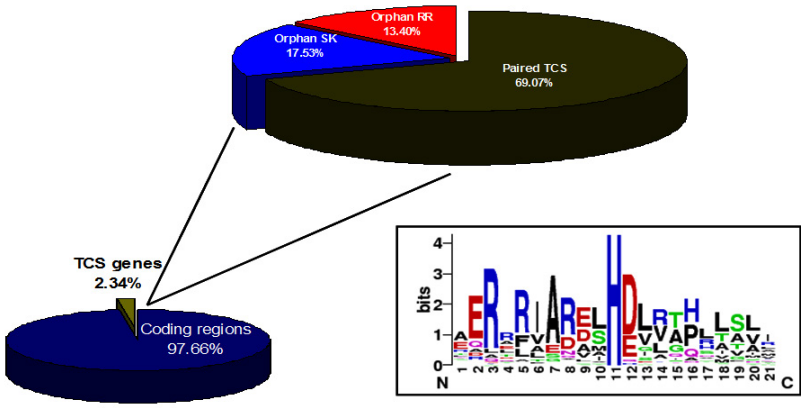

Fig. 2. Distribution of Two-component systems in genome of *S. coelicolor*. Box showing the highly conserved histidine. (http://www.p2cs.org) [147]

to grow on many different substrates. These enzymes are α-amylase, levansucrase, β-glucanase, xylanase and proteases. The production of all these enzymes is regulated by a TCS designated as DegS/DegU [98] where in *degS* encodes a sensor kinase and *degU* encodes for a response regulator. Deletion of *degS* and *degU* genes did not lead to variation in the phenotype. However, deletion led to the reduced production of these degradative enzymes [98]. A different class of mutations were also identified in *degS* and *degU* which led to the hyper production of degradative enzymes [99]. DegU which had threonine 98 mutated to isoleucine, valine 131 mutated to leucine displayed strong phosphorylation signals when compared to wild type DegU protein [100]. This indicated that DegU also has autophosphatase property where amino acid residue threonine 98 and valine 131 play a very significant role.

Proteins, apart from sensor kinases and response regulators, are known to be involved in the signal transduction processes [84] where a small protein protects the dephosphorylation of response regulator. Interestingly in the case of DegU there also exists a protein designated DegR which stabilizes the phosphorylated DegU. DegR protects dephosphorylation of DegU however the exact mechanism still remains unclear [101]. TCS involved in extracellular protease production was also identified in *Staphylococcus aureus*. ArlS/ ArlR TCS which not only regulates protease production, but also plays a significant role in virulence mechanisms of *S. aureus*. ArlS/ArlR TCS also functions as a regulator of peptidoglycan hydrolase activity as well as in bio-film formation [102].

Regulation of chitinases

Chitinase production in bacteria is regulated by a repressor/inducer system in which chitin or products of chitin degradation act as inducers. Experiments on cultures without carbon source to prevent catabolite repression revealed that N-acetylglucosamine is the best inducer of chitinase [103]. Induction of chitinases is substrate specific and not induced by pectin, xylan or cellulose. *Streptomyces* have a remarkable ability to utilize chitin as a source of carbon by producing multiple chitinases. Regulation of chitinases in *S. lividans* happens at the transcriptional level, chitin induces the production of chitinases and combination of chitin and glucose represses their production [104].

Catabolite repression of chitinase and other genes involved in utilization of carbon sources was identified in *ccrA1* mutant of *S. coelicolor*. Glucose repression of chitinase (*chi63*) production was abolished in a *ccrA1* mutant of *S. coelicolor* indicating its role in regulating *chi63* production [105]. The role of glucose kinase in glucose repression of chitinases has been established in *S. lividans* by introducing *glkA* gene from *S. coelicolor* into *S. lividans* G015 mutant which lacked glucose repression capabilities [106]. Interestingly, glucose repression of chitinase *chi63* promoter is independent of glucose kinase *glkA* [107]. DNA binding protein Cpb1 which had affinity towards chitinase promoters was purified from intracellular proteins of *S. lividans* by affinity purification [108]. This protein exhibited ability to bind chitinase promoters indicating its possible role in regulation. Disruption of *cpb1* gene provided a partial relief from glucose repression of chitinase production [108].

Reg1, a DNA binding protein identified in *S. lividans* has a helix-turn-helix motif (HTH) in the N-terminus. Disruption of *reg1* gene relieved the carbon catabolite repression of both ±-amylases and chitinases indicating the involvement of *reg1* [109]. *Streptomyces* in which *reg1* gene was disrupted lost the capability of chitin mediated induction of chitinases [109] indicating the role of *reg1* in induction of chitinases. Above mentioned studies indicated that more than one mechanism of glucose repression operates in *Streptomyces spp*. [107]. Presence of direct repeats has been reported in the promoters of chitinase genes from many *Streptomyces spp*. [1, 15, 110]. Partial characterization of *S. plicatus chi63* and *chi35* promoters identified that a single base pair substitution resulted in a strain which produced chitinase even in the presence of glucose. This mutant produced chitinase constitutively even in the absence of chitin [110]. Further work on *chi63* promoter has identified regions which influence both glucose repression as well as chitin induction [111]. Chitinase (*chiC*) regulated by a quorum-sensing system was identified for the first time in the case of opportunistic pathogen *P. aeruginosa* [112].

Chitinases regulated by TCS

Chitinases are enzymes which degrade chitin which is a homopolymer of β-1, 4-N-Acetyl-

D-glucosamine, one of the most abundant biopolymers on earth [112]. Chitinase production is constitutive and their production is enhanced by the presence of chitin. N-Acetyl glucosamine, the product of chitin breakdown is utilized by the bacterium as a source of carbon and nitrogen [113].

Expression of chitinases is regulated in the producing bacteria by means of catabolite repression and substrate induction. Two-component systems regulating chitinases have been reported from few *Streptomyces sp*. [113-116]. A hybrid TCS sensor kinase regulates chitinase production in *Vibrios* [117]. Two-component system involved in regulation of chitinase was first identified in *S. thermoviolaceus* and subsequently in *S. coelicolor* based on sequence homology to TCS from *S. thermoviolaceus*. Both these TCS not just share a high sequence homology but also share functional similarity with each other [53]. *S. peucetius*, well know producer of anti-cancer drugs doxorubicin and daunorubicin can degrade chitin and utilize for its growth effectively[1]. Chitinase production in *S. peucetius* is negatively regulated by ChiS/ChiR two-component system [118].

Allosamidin a family 18 chitinase inhibitor is produced by *Streptomyces sp.* AJ9463. Unlike other *Streptomyces*, this bacterium also produces chitinases. *Streptomyces sp.* AJ9463 *chi65* is regulated by two-component system which functions in very unique manner. Allosamidin can activate the transcription of *chi65* mediated by Chi65S/Chi65R TCS. Although allosamidin is involved in the transcriptional regulation it cannot activate on its own and it requires the presence of N, N"-diacetylchitobiose [116, 119]. Hybrid sensor kinase involved in regulating the production of chitinase was first reported in *Vibrio furnissi* and *Vibrio cholerae*. This hybrid sensor kinase (ChiS) controls the expression of approximately 50 genes of which many are involved in degradation of chitin [117].

Post translational modification of Chitinases

Glycosylation

Glycosylation, the post-translational modification of proteins by carbohydrates has long been recognized as a key strategy to influence structure and function of proteins in eukaryotes [120]. For long it was believed that glycosylation occurs exclusively in eukaryotes, however this has been challenged by the identification of glycoproteins from prokaryotes [121]. The surface layer (S-layer) glycoprotein of *Halobacterium halobium* (*Salinarum*) was the first prokaryotic glycoprotein to be identified [121]. Identification of glycoproteins is most often based on their aberrant migration in SDS-PAGE. However, glycosylation of proteins can also be detected by oxidation of carbohydrates mediated by periodic acid and condensation of generated aldehyde by Schiff's base with a chromogen or an indicator enzyme. Oxidized sugar could also be detected by digoxigenin (DIG), DIG in turn is detected by Anti-DIG antibodies [122]. Lectin based identification of glycoprotein's is also a feasible option. The enzymatic removal of glycan from the protein can identify whether glycosylation is N- linked or O-linked [2, 123].

Tunicamycin, an inhibitor of N-linked glycosylation has been used to study the functional significance of glycosylation in Sf9 (*Spodographa frugiperda*) cells expressing a chitinase from a tick *Haemaphysalis longicornis* [24]. Tunicamycin along with enzymatic removal of glycans can be used to identify the nature of glycosylation [2]. Identification of glycans by mass spectrometry involves the separation of glycan either by enzymatic or by chemical methods [120]. N-linked glycans are removed by N-glycosidases such as PNGaseF. O-linked glycosylation can be removed both by chemical method such as reductive alkaline b-elimination and by enzymatic method using O-glycanases [2, 123]. A few chitinases that have been found to be glycosylated are ChiA from *Cellulomonas uda* [18], ChiC from *S. griseus* [21], CHT1 from *Haemaphysalis longicornis* [24] and (human analog of chitinases) chitotriosidase produced by macrophages in humans [2]. The functional significance of glycosylation in chitinases is not known however, in the case of cellulases glycosylation plays a significant role in binding to crystalline substrate and also protects the enzyme from cleavage by proteases [25].

Proteolytic processing

Streptomyces are the major producers of chitinases in soil. Multiple chitinase genes have been identified in the genome sequences of *S. coelicolor* [9], *S. avermitilis* [10] and *S. griseus* [11]. Several isoforms of chitinases have also been identified and they could have been the products of different genes or the result of post-translational proteolytic processing. This assumption was

first made for the multiple chitinases in *Bacillus circulans* [12]. Post-translational proteolytic processing has also been observed in the case of chitinases of *Serratia marcescens* [124, 125] and also in the case of cellulases of *S. reticuli* [126]. Among *Streptomyces*, proteolytic cleavage of chitinases was first observed in *S. olivaceoviridis* where in 70 kDa chitinase served as a precursor for 30 kDa and 20.5 kDa chitinase. Chitinase of 20.5 kDa is derived from 30 kDa or 70 kDa chitinase upon proteolytic cleavage [19]. Even though both 30 kDa and 20.5 kDa chitinases had the same active site 20.5 kDa chitinase shows reduced specific activity [19].

Chitinases which have originated from the same precursor by proteolytic cleavage also show differences in binding and substrate specificities [127]. In the case of *S. marcescens* chitinase both precursor and mature chitinase show similar specific activities and optimal reaction temperature [125]. Family 19 chitinase C-1 identified in *S. griseus* was also derived from Chitinase C by proteolytic cleavage [128].

Origin of multiple chitinases from the same precursor by proteolytic cleavage is not just limited to bacteria. Chitinases of plants such as *Phaseolus vulgaris* L. cv Saxa namely PvChiE, PvChiF and PvChiG were all derived from PvChi4 by differential proteolytic cleavage [129]. Human analogue of chitinases namely chitotriosidase secreted by macrophages exhibits multiple forms of chitotriosidases both by alternative splicing of mRNA and also by proteolytic cleavage [2]. *S. olivaceoviridis* produces an autocatalytic chitinase [130] which has a lysine C-endoproteinase in the C-terminus. This protein remains as a 92 kDa protein in the presence of protease inhibitors. However, 70 kDa and 22 kDa fragments are produced on removal of the protease inhibitors. The resultant 22 kDa fragment has proteolytic activity [130].

Chitinases- importance and application

Chitinases are enzymes which cleave the glycosidic linkages of chitin to generate low molecular weight oligosaccharides. Based on the mode of cleavage chitinases can be broadly grouped into two categories. The endochitinases which cleave randomly and generate chito-oligosaccharides of various sizes and exochitinases cleave from the non-reducing end of chitin microfibril [29]. Based on the sequence similarity chitinolytic enzymes can be grouped into families 18 and19. Family 18 chitinases are the most diverse of the three families and are found in bacteria, fungi, viruses, animals and plants. Family 19 chitinases are abundant in plants [129] and a few *Streptomyces* [3]. Family 18 and family 19 chitinase do not share sequence similarity, have different structures and molecular mechanisms indicating that these enzymes are likely to have evolved from different ancestors [20].

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