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Advances in pH-Signaling Transcription Factor pacC in Fungi

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Review Article

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ABSTRACT

In order to maintain the intracellular acid–alkaline balance, growth and metabolism, microorganisms have evolved a pH pathway that includes the *pacC* gene and pal genes, to respond to and transmit ambient pH. The *PacC* protein is a key regulator in this pathway, initially found in *Aspergillus nidulans* and later found in a variety of fungi. The transcription factor *PacC* includes three zinc fingers and three regions A–C, and has an active form, a short-chain polypeptide formed through two successive hydrolysis processes. This active form can promote expression of acidic genes, inhibit expression of alkaline genes, and regulate growth, toxin production and pathogenicity. Phenotypic variation in different *pacC* mutants indicates that the regulatory role of *PacC* is not entirely consistent in different strains. This review summarizes the above mentioned points and discusses the different functions of *PacC* in a variety of fungi, provides the mechanism of *PacC* response to the external environment and the chain response of other pathways regulated by *PacC*.

INTRODUCTION

External pH is an important environmental factor affecting the growth of microorganisms. The tolerance of the surrounding pH is an important manifestation of microbial adaptation ability. Vital activity and microorganism metabolism are closely related to external pH. Most microorganisms adapt to a pH range of 4.5–9, with optimum pH of 6.5–7.5. In addition, environmental pH has a regulatory role in spore germination and spore production [1-5]. At present, there is a general view that the external pH signal has a regulatory impact on the pathogenicity and toxin production of pathogenic fungi. The pH can regulate the secretion of pectin lyase [6] and so the pathogenicity is closely related fungi producing pectin lyase. By adjusting pH to better infect hosts, a variety of pathogens such as *Colletotrichum spp.*[6,7] and Alternaria alternate [8] can produce alkaline substances to alkalize host tissue, and *in vivo* pH will rises with further with infection of the host. Some pathogens such as *Sclerotinia sclerotiorum*, *Botrytis cinerea* and *Penicillium spp.* can acidify tissue cells by producing an acidic substance or the use of ammonia [9-11]. The surface of barley and rice was originally weakly acidic, but after pathogen infection the pH changed to alkaline indicating that infection affected pH of the host [5]. These phenomena indicate the adaptation of fungi to the external environment.

In order to adapt to the different pH conditions of the external environment, fungi have evolved a signal transduction pathway that responds to ambient pH. This process allows microbes to adapt to changes in external pH environment, and ensures growth of fungi [12]. Infection by pathogenic fungi in animals and plants requires responsing external pH. Many fungi respond to external pH through inducing changes in gene expression, which may controlled by the pH regulation pathway. The mechanism of signal induction and transmission to environmental pH in fungi, such as *Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus nidulans*, has been extensively studied [13-15]. The pathway mediated by the zinc finger transcription factor *PacC* is the most well studied pH signal transduction pathway.

PacC TRANSCRIPTION FACTOR

External pH can have a toxic effect on many microbes. In order to maintain an intracellular acid–alkaline balance and well-balanced growth, microorganisms have built a system regulated by outputs such as permeability and enzyme secretion. In 1965,

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the *PacC* regulation function on pH was first discovered in *A. nidulans* [16]. The acid or alkaline phosphatase (secreted under acidic or alkaline conditions) deletion mutants was isolated in A. nidulans. Wherein, *palA*, *palB, palC, palD, palE* and *palF* were related to regulation of alkaline or acid phosphatase.

In acidic conditions, acidic genes were activated nor alkaline genes as well as *PacC* gene were down-regulated; in alkaline conditions, *PacC* activates transcription of alkaline-expressed genes (including *pacC* itself) and represses transcription of acid-expressed genes in the presence of a signal mediated by the products of the pal genes [17]. *PacC* encoded a transcription regulatory protein related to pH, was successively identified in *Aspergillus niger* [18], *Aspergillus ochraceus* [19], *Beauveria bassiana* [20], *Colletotrichum gloeosporioides* [6], *Fusarium oxysporum* [21], *Ganoderma lucidum* [22], *Magnaporthe oryzae* [5], *Metarhizium robertsii* [23], *Penicillium chrysogenum* [24], *Penicillium digitatum* [25], *Penicillium expansum* [26], *Trichoderma harzianum* [27] and so on. PacC was highly expressed under alkaline conditions. With decreasing pH, the expression level of pacC decreased [5,6,18-27] – this property is regulated by the active ingredient of PacC.

The DNA binding sites (DBD) of PacC contains three Cys2His2 zinc fingers, and PacC fusion protein can bind DNA with a core consensus GCCARG [17,28]. Downstream of the DBD, PacC included three functional domains A–C, within domain C near the C-terminal [29]. The amino acid sequences of three zinc finger domains in the nine strains (*A. nidulans, A. niger, A. ochraceus, A. fumigatus, P. digitatum, F. oxysporum, F. graminearum, S. cerevisiae and S. sclerotiorum*) had high homology, particularly in the sequences of the binding moiety of DBD and the zinc fingers 2 and 3 [19]. It is forecast that the center of PacC binding sites are consistent in the different strains.

The proteolysis of *PacC* is important to the regulation function of gene expression in an alkaline environment [10]. Originally, *Aspergillus PacC* is itself an alkaline-expressed gene, subject to autogenous transcriptional activation, amplifying the alkaline ambient pH signal [30,31]. PacC existed in three conformations: full-length, transitional and processed forms [29]. The structure of full-length *PacC* contained two forms: open and closed. Under acidic conditions, PacC exists as total-length PacC72 (72 kDa) including 678 amino acid residues with enclosed structure. The interaction of three functional domains of PacC72 can prevent protease hydrolysis. In neutral to alkaline conditions, through two-step sequential proteolysis [30-32], PacC72 assumes the active form, which can promote alkaline gene expression [13, 17] and inhibit acidic gene expression [17,33]. The protein is active under alkaline conditions but is inhibited under acidic conditions [13,17,32]. Under alkaline conditions, PacC forms an open structure, removing about 180 amino acid residues in the C-terminus, forming polypeptide PacC53 (53 kDa) [12,30]. Then, under the function of protease catalyzing [34], more than 245 C-terminal residues are removed from PacC53 and the three zinc fingers are completely preserved. PacC53 turn into a shorter form (27 kDa): PacC27, an active form [29,30]. This step does not depend on external pH. The active structure finally enters the nucleus and regulates a series of gene expression which can affect growth and pathogenicity.

The pH regulatory pathway in *A. nidulans* includes seven factors: PacC, PalA, PalB, PalC, PalF, PalH and PalI [12,35], among which PacC plays the leading role. Six pal genes are crucial for transforming PacC from the inert to the active form [18,25]. The Pac/Pal system mediates a rapid and effective response to alkalinisation, switching genes on or off as appropriate. The pal genes are all involved in transcription under alkaline conditions, and the response to alkaline conditions requires seven genes work together [15,36]. Although necessary, Pal proteins are insufficient to transmit the signal, which additionally requires ESCRT-I, II and Vps20 with Snf7 in ESCRT-III. The model of the endosomal sorting complex required for transport (ESCRT)-mediated pH signaling at the plasma membrane was proposed [37-39]. PacC27 only exists in the nucleus and PacC72 is present in the cytoplasm, while PacC53 occurs in both nucleus and cytoplasm [40].

However, fungi also grow in acidic environments and normally prefer to do so. *A. nidulans* can grow in media of pH values as low as pH 2.0 [41]. To adapt to acidic conditions, fungi must be able to control, even switch off, the alkaline ambient pH response. Repression of acid-expressed PalF, specifying the Pal pathway arrestin, probably by PacC27 and/orPacC53, prevents an escalating alkaline pH response [42]. PacX, a new component of the pH regulatory machinery, is required in pacC repression. pacX mutations suppress PacC processing recalcitrant mutations, in part, through derepressed PacC levels resulting in traces of PacC27 formed by pH-independent proteolysis [42].

MULTIPLE BIOLOGICAL FUNCTIONS OF PAcc IN FUNGI

PacC not only participates in the pH regulatory pathway, but also in oxidative stress, ion pressure, animal and plant pathogenesis, toxin production and food spoilage [35]. Regulation of PacC in spore production, toxin production and plant pathogenesis differs in various strains — some are positive and some are negative — but it can inhibit cell growth under alkaline conditions in all strains. PacC genes and their functions in different strains were summarized in Table 1.

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Table 1: *PacC* genes and their functions in different strains.

Strain	Gene	Gene bank No.	Length of amino acid	Characters
Acremonium chrysogenum	PacC	Q96X49.1	621	Related to biosynthesis of cephalosporin C [56].
Alternaria alternata	PacC	XP_018388469	594	Regulate alternariol production and had affect the capacity to colonize tomatoes [8].
Aspergillus fumigatus	PacC	Q4WY67.1	676	Regulate growth, secretory protein expression under alkaline and infection and biosynthesis of gliotoxin, mutant reduced sensitivity to cell wall hydrolytic enzymes [43].
Aspergillus giganteus	PacC	Q5XL24.1	678	Expression increased as pH and time increased, negative activity to β-Galactosidase [57].
Aspergillus nidulans	PacC	Q00202.1	678	Positive regulate spore production and growth, promote alkaline genes, acid phosphatase, inhibit the expression of alkaline phosphatase and acid gene pacA [13, 17], can modulate extracellular enzymes xylanase gene xlna and xlnb [52] and cellulase genes [51], positive regulation to pathogenic, penicillin and ST [48].
Aspergillus niger	PacC	Q00203.1	667	High expression under basic conditions, not expressed under acidic condition, but also related to expression of acid hydrolases [18].
Aspergillus ochraceus	AoPacC	g6246.1	681	Regulate mycelium growth and sporulation, have a positive in ochratoxin A production and pathogenicity [19].
Beauveria bassiana	BbPacC	AFP94095.1	590	Loss of pacC resulted in an alkaline pH-dependent decrease in growth and pH-dependent increased susceptibility to osmotic (salt, sorbitol) stress and SDS [20].
Botrytis cinerea	BcPacC	-	-	The pathogenicity of the mutant was altered on plants exhibiting a neutral pH and not on plants with acidic tissues [58].
Canidia albicans	RIM101	Q9UW14.2	661	Is required for host-pathogen interactions [14]. Inducing alkaline response gene expression and repress acidic response gene expression [59].
Clonostachys rosea	PacC	AC006060.1	602	Expression increased With the pH increased, growth under alkaline, mycelium, spore production, pathogenicity, expression of virulence factors serine protease Prc reduced in mutants [60].
Colletotrichum acutatum	PacC	ABL96218.1	590	Increase acid phosphatase and reduce alkaline phosphatase. Mutant sensitive to alkaline conditions, production of spores and appressorium increase, spore germination and pathogenicity fall, improve pectinase, polygalacturonic acid and lipolysis activity [46].
Colletotrichum gloeosporioides	Pac1	ABR37627.1	584	Expression stronger under alkaline [6], positive regulation its downstream gene PELB expression, pectinase PL secretion and pathogenic [7].
Colletotrichum lindemuthianum	Pac1	AFU83185.1	582	Expression increased with the pH increased, positively regulate the growth and sporulation under alkaline conditions, the mutant pathogenic weakened [61].
Epichloë festucae	PacC	EfM3.009480	591	Regulate the sensitivity to salt-stress but not affect growing under alkaline conditions [47].
Exophiala dermatitidis	PacC	ACB12920.1	646	Expression increased with the pH increased, deletion mutants grown is affected in normal medium, spores swelling increases [62].
Fusarium graminearum	Pac1	AD060821.1	619	Expression increases with pH and time increasing, mutants grow slower under alkaline conditions, mycelium reducing, growth rate decreased, increased sensitivity to hydrogen peroxide, toxin levels than the wild-type [63].

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Fusarium oxysporum	PacC	Q870A3.1	609	Expression increases with pH increasing, showing negative regulation to pathogenic, related with neomycin and hydrogen peroxide [21].
Fusarium verticillioides	Pac1	Q873X0.1	613	FB1 production increased, spores lower production in mutant, cannot grow in alkaline conditions [45].
Metarhizium robertsii	MrpacC	XP_007822227.1	592	Regulate sporulation, insect cuticle penetration and immune evasion [23].
Penicillium chrysogenum	PacC	Q01864.2	643	More significant expression under alkaline conditions, and related to penicillin [24].
Penicillium digitatum	PacC	AFS18474.1	641	Associated with pathogenicity and growth on alkaline conditions, mutants increased sensitivity to monovalent cations [25].
Penicillium expansum	PePacC	GCA_001008385.1	-	Plays pivotal roles in virulence and patulin biosynthesis [26].
Saccharomyces cerevisiae	RIM101	P33400.2	625	Expression increased under alkaline conditions, promote alkaline gene expression, and inhibit acid gene expression [64].
Sclerotinia sclerotiorum	Pac1	Q9P413.1	625	Expression increases with increased of pH and time [65].
*Trichoderma harzian*um	PacC	ABK60115.1	620	Expression increases with pH increasing, can regulate expression of chitinase, proteases, glucose permease, cell wall protein [27].
Trichoderma reesei	Pac1	EGR50864.1	622	Expression increases with increased of pH, promote expression of alkaline gene, inhibition of gene expression of acid gene [66].
Trichophyton rubrum	PacC	Q9C1A4.2	758	Positive regulate growth and pathogenic [54].

PacC related with fungi growth and spore production under alkaline conditions

The *A. fumigatus pacC* mutants grow slower under alkaline conditions than acid or neutral conditions, with changes in mycelium structure [43], showing sensitivity to alkaline conditions [1,21,44]. Strains missing pacC gene do not grow normally under alkaline conditions, but covering mutant can alleviate this phenomenon. Loss of PePacC resulted in an obvious decrease in growth and conidiation of *Penicillum expansum* cultured in both acidic and alkaline condition [26]. Loss of *pacC* in the insect pathogenic fungus *Beauveria bassiana* resulted in an alkaline pH-dependent decrease in growth [20]. The mycelium growth was inhibited under pH 4.5 and 10.0 in *Aspergillus ochraceus*, while the sporulation increased under alkaline condition. A reduction of mycelium growth and an elevation of sporulation was observed in *ΔAopacC* mutant [19]. The PacC also exhibits a regulatory function in spore production, but has different roles in different strains, some showing positive regulation in *Alternaria alternata, Fusarium verticillioides, Magnaporthe oryzae, Metarhizium robertsii* [5,8,23,45] and others negative regulation in *Aspergillus ochraceus* and *Colletotrichum acutatum* [19,46]. Alkaline pH was observed to induce conidiation in wild-type *Epichloë festucae*, but surprisingly deletion of pacC did not affect the ability of the mutant to grow under alkaline pH conditions [47].

PacC associated with toxin production

The pacC is associated with toxin synthesis, but has different regulation roles in different strains. It can positively regulate penicillin production in *P. chrysogenum* and *A. nidulans* [24,48] and sterigmatocystin [49] in *A. nidulans*; and has been shown to inhibit toxin synthesis in other filamentous fungi, such as FB_1 produced by *F. oxysporum* [21]. The *ΔPePacC* mutant lost the ability of patulin production at pH values above 6.0 because expressions of all the genes in patulin cluster were significantly down-regulated [26]. During colonization of apple fruit *in vivo* and growth in culture, *P. expansum* secretes pH-modulating D-gluconic acid and ammonia. Ammonia activates pacC and patulin accumulation in an acidic environment [50]. The expression of *AopacC* increased with the elevated pH, and deleting *AopacC* dramatically decreased ochratoxin A production and biosynthetic genes *Aopks* expression in *A. ochraceus* [19]. HogA and PacC regulated alternariol biosynthesis in *Alternaria alternate*. Mutant strains of *A. alternata*, not able to produce alternariol, had a greatly reduced capacity to colonize tomatoes, a high water activity and a low pH tissue, compared to the wild type [8].

Regulation of gene expression and enzyme secretion by PacC

In *A. nidulans*, several genes encoding cellulolytic enzymes are regulated by PalI-PacC-mediated pH signaling [51], and pacC can regulate the expression of extracellular enzymes xylanase genes xlnA and xlnB [52]. There is acidic and alkaline phosphatase activity under neutral conditions in *Aspergillus nidulans* wild type, yet the pacC mutant lost acid phosphatase activity while retaining alkaline phosphatase activity [48]. Such phenomena are also found in other strains and show that PacC can promote acid phos-

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phatase and inhibit alkaline phosphatase production [46]. Furthermore, pacC homologous gene pac1 in *Trichoderma harzianum* can adjust the expression of chitinase, proteases, glucose permease and cell wall protein [27]. MrpacC transcriptional control of chitinase and glucosyl-transferase genes in *Metarhizium robertsii* [23].

Involvement of PacC in regulation of pathogenicity of fungi

PacC has a very important role in virulence, and may be directly involved in the infection pathway or regulating factors in these pathways. PacC is often positively correlated with pathogenicity. However, the roles of PacC is different in different fungi. Pathogenicity in pacC mutants is reduced [53] and other genes in the pH pathway mediated by PacC can also regulate pathogenicity in Aspergillus. It is difficult to determine whether the active N-terminal of PacC or PacC itself plays a role in virulence. Moreover, expression of pacC increased if pectin was used as the sole nitrogen source, probably because PacC improved pectin utilization by regulating cell wall degrading enzyme gene pdpg2. Pectinase is related to pathogenicity, so PacC may associated with pathogenicity by regulating encoding enzymes related to pathogenicity [25]. This is similar to the regulation mechanism of Pac1 found in other microorganisms, such as *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* [6,7,10]. Similarly, it was found that PacC could also regulate the activity of glycoproteins and lysozymes [5], both of them are associated with pathogenicity. The *ΔAopacC* mutant in *Aspergillus ochraceus* exhibited attenuated infection ability toward pear fruits [19]. The virulence of the *ΔPePacC* mutant in *Penicillum expansum* was obviously reduced in pear and apple fruits, and *PePacC* play an important role in pathogenesis via mediating a known virulence factor glucose oxidase and new virulence factors, such as calreticulin and sulfate adenylyltransferase [26]. In other microorganisms, such as *C. albicans, Trichophyton rubrum*, *Colletotrichum acutatum* and *P. digitatum*, PacC showed a positive regulation function in pathogenicity [14,22,46,54]. Moreover, PacC also related with the biocontrol of antagonismin in *Trichoderma virens* [44]. In contrast, PacC was negatively correlated with pathogenicity in other organisms – in *Fusarium oxysporum*, pathogenicity of the pacC mutation was higher than in wild type [21]. PacC had only minor effects on virulence in the insect pathogenic fungus *Beauveria bassiana* [20]. Thus the regulatory mechanism of PacC in infection ability differs among different fungi.

Other functions

Furthermore, *pacC* mutants also exhibit sensitivity to monovalent cations [25], antibiotics [21, 55-66] and hydrogen peroxide [21], suggesting that PacC is also involved in oxidative stress and ionic stress, and is related to antibiotic resistance. PacC regulates salt tolerance and expression of the *enaA* gene coding for a $Na^+(K^+)$-ATPase in *Epichloë festucae* [47]. Loss of *pacC* in *Beauveria bassiana* resulted in pH-dependent increased susceptibility to osmotic (salt, sorbitol) stress and SDS [20]. MrpacC regulates insect cuticle penetration and immune evasion in *Metarhizium robertsii*, deletion of *MrpacC* impaired fungal responses to ambient pH and salt/metal challenges but not osmotic stress [23].

CONCLUSION

Through the pH pathway mediated by PacC, the singal of pH changes transmit to the cell nucleus and cause a series of changes of gene expression and extracellular enzyme secretion, so that microorganisms can better adapt to external pH. It has been found that PacC is involved in the synthesis of a variety of toxins, pathogenicity and antagonistic regulation of biocontrol microorganisms. Exploring transcription factors regulating the synthesis of toxins will provide a new theory to explain the molecular biosynthesis mechanism of toxins, especially some mycotoxins that have strong carcinogenic toxicity, are widely found in food and feed, and seriously endanger food security. The PacC can regulate the expression of a variety of hydrolytic enzymes associated with pathogenicity, but how these enzymes regulate virulence factors, and what the factors effect is not clear. In-depth analysis of these regulatory factors, defining the role of upstream and downstream regulators and studying molecular metabolic mechanisms of pathogenic microorganisms can provide a theoretical basis for prevention and management of pathogens. Biocontrol microorganism have important economic value. Studying the resistant mechanisms and regulatory pathways of antagonistic microorganisms can help in understanding the interactions between antagonistic microorganisms and pathogens.

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