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Review

The effects of salicylate on bacteria

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Abstract

Salicylate and related compounds, such as aspirin, have a variety of effects in eucaryotic systems and are well known for their medicinal properties. Salicylate also has numerous effects on bacteria, yet only a handful of individuals within the scientific community appreciate these findings. From a bacterial viewpoint, growth in the presence of salicylate can be both beneficial and detrimental. On one hand, growth of certain bacteria in the presence of salicylate can induce an intrinsic multiple antibiotic resistance phenotype. On the other hand, growth in the presence of salicylate can reduce the resistance to some antibiotics and affect virulence factor production in some bacteria. This review provides an overview of the effects salicylate has on various bacterial species.

Keywords: Salicylate; Bacteria; Eucaryotes

Contents

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1. Introduction

Salicylate is one member of a large group of pharmaceuticals referred to as non-steroidal anti-inflammatories and is the active component of the analgesic aspirin [1]. Salicylate is found ubiquitously in plants and has been used for medicinal purposes since antiquity [1]. Today, ~16 000 tons of aspirin (acetyl-salicylate) are consumed every year in America generating sales of ~US$2 billion for these over-the-counter painkillers [2]. Although widely used as an analgesic, it has become apparent that salicylate has a diverse range of effects in both eucaryotic and bacterial systems. This review concentrates on the effects of salicylate in bacteria.

2. Effects of salicylates in eucaryotes

Aspirin, the most commonly administered form of salicylate, can reduce the inflammation and pain associated with many illnesses [2], with its anti-inflammatory properties largely attributed to inhibition of prostaglandin synthesis [3–5]. Prostaglandins are small membrane associated fatty acids that act as local hormones mediating the control of a number of cellular processes including inflammation [6]. Aspirin inhibits cyclooxygenase-1 [7] an enzyme necessary for prostaglandin synthesis (Fig. 1). This results in a reduction in prostaglandin levels and hence, inflammation. Aspirin is also considered to offer some protection against coronary heart disease [2], due in part to inhibition of the prostaglandin thromboxane A_2, a potent platelet aggregator. Aspirin also reduces apolipoprotein production, high concentrations of which are known to contribute to vascular disorders [8].

Many of the proteins associated with the inflammatory response are induced by transcription factor nuclear factor-κB (NF-κB) [9–12]. NF-κB is held inactive in the cytosol by the protein IκB and under appropriate stimuli is released from this interaction and translocates to the nucleus. Salicylate inhibits the activation of NF-κB by interfering with the pathway that modifies or degrades IκB, preventing the release and translocation of this transcription factor into the nucleus [13] (Fig. 1). It is thought that salicylate interferes with a pathway that modifies or degrades IκB preventing the release of NF-κB [13]. The presence of salicylate at high concentrations can also prevent transcription of two inducible NF-κB promoters within the long terminal repeats of the human immunodeficiency virus-1 [13–15].

The elevated temperatures associated with inflammation may activate heat shock transcription factor 1 (HSF1) [16]. When this transcription factor is activated it binds to heat shock gene promoters, increasing their expression (Fig. 1). Salicylate can induce the binding of HSF1 to heat shock promoters but this event alone does not result in increased expression of heat shock genes [16,17] (Fig. 1). Salicylate and heat can however act synergistically to increase heat shock gene transcription [17]. Therefore it is possible that the anti-inflammatory effects of salicylate involve an alteration of the heat shock response [17].

Salicylate has been shown to have chemopreventative activity against colorectal and esophageal cancers [18–20]. It has also been shown that salicylate induces apoptosis (programmed cell death) in a number of leukemia cell lines [21,22]. This is achieved through the proteolytic activation of caspase-3, a protease involved with the onset of apoptosis and the inhibition at the transcriptional level of MCL-1, an antiapoptotic factor [22] (Fig. 1).

In addition to its effects in mammalian cells, salicylate is also involved with systemic acquired resistance (SAR) in plants. SAR is reliant on the expression of pathogenesis-related (PR) genes which are activated following exposure to a pathogen [23] leading to broad spectrum protection not only against the original invading pathogen but also unrelated pathogens [24,25]. Salicylate induces expression of PR proteins and this leads to SAR in tobacco plants [26–28] (Fig. 1). This salicylate-induced response may therefore help explain the ubiquity of salicylate in plants.

3. Effects of salicylate on bacteria

Somewhat surprisingly, salicylate induces a number of morphological and physiological alterations in bacteria. Growth of bacteria in the presence of salicylate can: (a) induce an intrinsic multiple antibiotic resistance phenotype; (b) reduce the resistance to some antibiotics; and (c) affect the production of various factors involved with bacterial virulence. These effects are induced by concentrations of salicylate that do not grossly affect bacterial growth rates suggesting that they are specifically induced by salicylate.

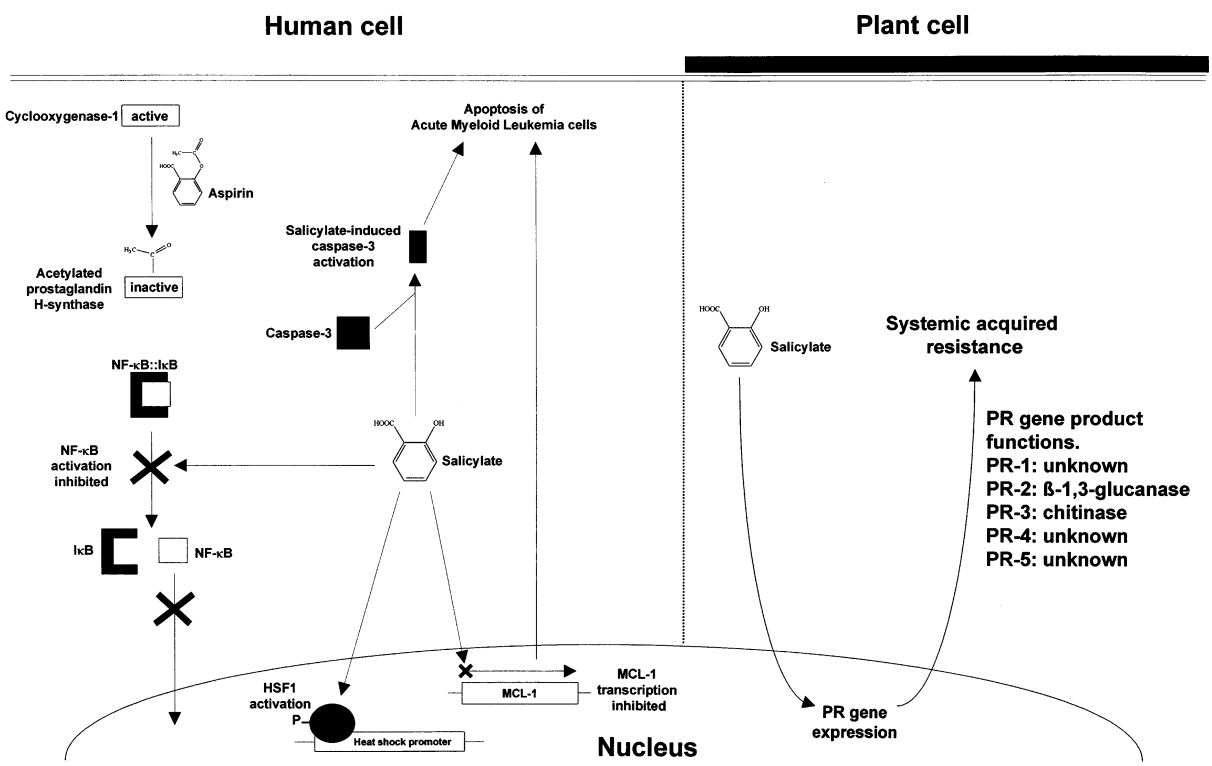

Fig. 1. The effects of salicylate in a generalized human and plant cell.

Table 1
Salicylate-induced antibiotic resistances and bacterial membrane protein alterations

Organism	Increased antibiotic resistance phenotype	Known membrane protein alterations		References
		Increase	Decrease	
Escherichia coli	Ampicillin, cephalosporins, chloramphenicol, nalidixic acid, fluoroquinolones, tetracycline, thiolactomycin	AcrAB, TolC	OmpF	[30–33]
Salmonella typhimurium	Chloramphenicol, enoxacin	?	?	[34]
Klebsiella pneumoniae	β-Lactams, clindamycin, norfloxacin, tetracycline	–	Porin A, Porin B	[35,36]
Pseudomonas aeruginosa	β-Lactams, carbapenums, quinolones	OprN	OprD, OprJ	[37,38]
Burkholderia cepacia	Chloramphenicol, ciprofloxacin, trimethoprim	–	OpcS	[39]
Serratia marcescens	?	OmpC	OmpF	[40]
Staphylococcus aureus	Fusidic acid, fluoroquinolones	?	?	[41,42]

3.1. Effects of salicylate on bacterial antibiotic resistance

In general, bacterial antibiotic resistance arises due to the acquisition of exogenous genes or as a result of mutation(s) in chromosomal genes that mediate resistance to one or a number of closely related antibiotics [29]. However, even strains considered susceptible to antibiotics exhibit a degree of intrinsic resistance to the effects of these compounds. Growth of several bacterial species in the presence of salicylate leads to an increase in their levels of intrinsic antibiotic resistance [30–42] (Table 1). In most cases, salicylate-induced antibiotic resistance has been attributed to an alteration in the synthesis of membrane proteins leading to a reduction in drug accumulation (Table 1, Fig. 2).

When grown in the presence of salicylate the Gram-negative organism, *Escherichia coli*, exhibits increased resistance to multiple antibiotics including quinolones, cephalosporins, ampicillin, nalidixic acid, tetracycline and chloramphenicol [30,31]. Salicylate-induced multiple antibiotic resistance in *E. coli* is due in part to increased transcription of the *marRAB* operon (Fig. 2) [43]. Of the genes located within *marRAB*, *marR* encodes a negative regulator of the operon, and *marA*, encodes a transcriptional activator [44]. Both MarR and MarA are intimately involved with the salicylate-induced multiple antibiotic resistance mechanism in *E. coli* [43]. MarA modulates the transcription of a number of unlinked loci constituting the *mar* regulon (for review see [45]) and expression of these genes results in a multiple antibiotic resistance phenotype. Salicylate inhibits the binding of MarR to *marO*, the operator region of the *mar* operon [46,47] which then leads to increased production of MarA and a decrease in antibiotic accumulation. This decreased accumulation is due to reduced production of outer membrane porins OmpF and possibly OmpC [48] and to a concomitant increase in the production of the multidrug efflux pump AcrAB [32]. The salicylate induced reduction in OmpF is due to increased transcription of *micF*, which encodes an *ompF* antisense-RNA [43,48]. Binding of the *micF* antisense-RNA with the *ompF* transcript prevents *ompF* translation (Fig. 2). OmpF and OmpC normally allow the passive diffusion of some antibiotics into the cell [49] while AcrAB, in conjunction with TolC [33] (Fig. 2), actively reduce the intracellular concentration of various drugs [32]. In *E. coli*, at least two other multidrug efflux pumps are also affected by salicylate. EmrR, the negative regulator

of the multidrug efflux pump operon *emrAB*, shares sequence similarity to MarR and transcription of the *emrAB* is inducible by salicylate [50]. Another locus, *emrKY*, encodes another efflux pump that is homologous to EmrAB and this to is inducible by salicylate, tetracycline and chloramphenicol [51]. The operons *emrAB* and *emrKY* may therefore also be involved with the salicylate-induced multiple antibiotic resistance mechanism in *E. coli*.

Salicylate associated increases in antibiotic resistance are seen in bacteria other than *E. coli*. When grown in the presence of salicylate, *Salmonella typhimurium* expresses increased resistance to chloramphenicol and enoxacin [34]. This increased resistance is due to the induction of the *S. typhimurium mar* regulon by salicylate [34]. *Klebsiella pneumoniae* also exhibits increased resistance to tetracycline, β-lactams, clindamycin and norfloxacin when salicylate is present in the growth medium [36]. This salicylate-induced effect is due to increased expression of a MarA homologue, RamA, and the reduced production of two porins [35,52] (Table 1).

Recently it was shown that salicylate induces increased phenotypic resistance to fluoroquinolones and fusidic acid in the Gram positive organism, *Staphylococcus aureus* [41,42]. The effects of salicylate on fusidic acid resistance levels in cell populations of fusidic acid-susceptible and -resistant strains of *S. aureus* are shown in Fig. 3. The presence of salicylate also increases the frequency at which susceptible *S. aureus* mutates to become fusidic acid resistant, or resistant *S. aureus* mutates to higher levels of resistance to the drug [42] (Fig. 3). Salicylate also increases the frequency at which susceptible *S. aureus* mutates to become fluoroquinolone resistant [41]. The mutations leading to fusidic acid and fluoroquinolone resistance occur at unrelated loci within the *S. aureus* chromosome [53,54]. Since salicylate has been re-

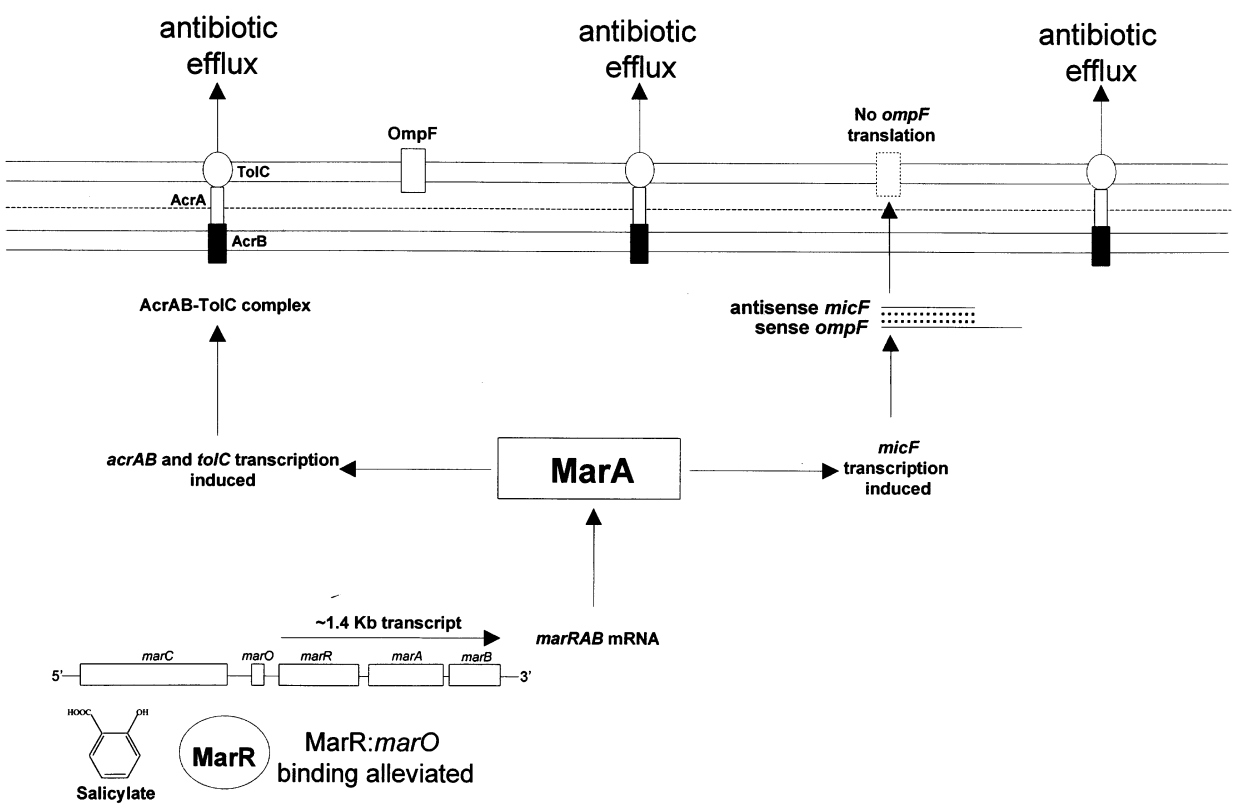

Fig. 2. The effects of salicylate on the *E. coli mar* operon and membrane physiology.

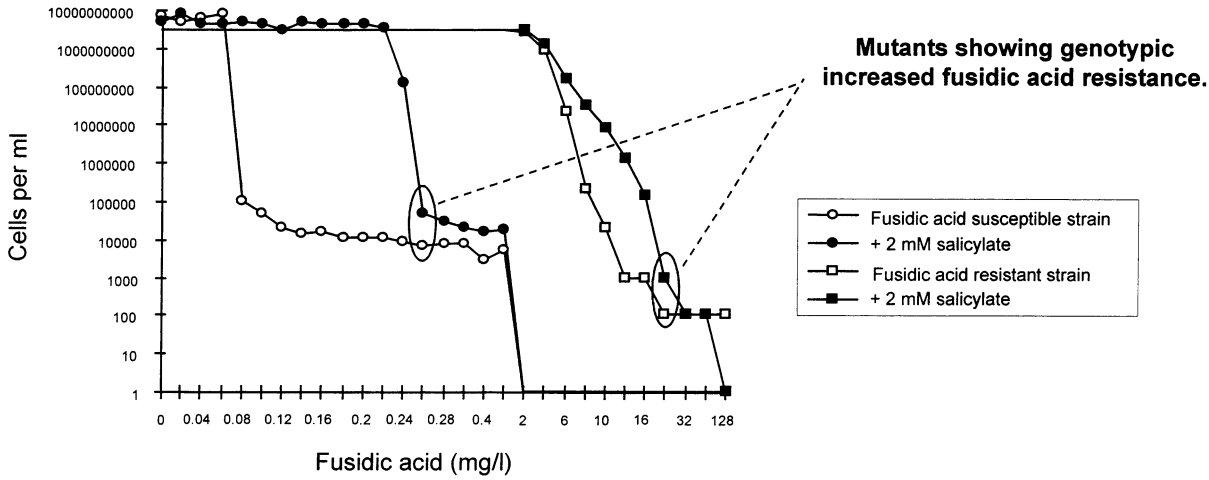

Fig. 3. Population analyses of fusidic-susceptible and -resistant strains of *S. aureus* performed with and without 2 mM salicylate.

ported to be nonmutagenic using the Ames test [55], the increased mutation frequencies observed in salicylate treated cells is probably due to salicylate-induced gene products. The strong selection pressure of antibiotic resistance may also influence the increased mutation frequencies observed.

3.2. Possible candidate genes involved with salicylate-induced multiple antibiotic resistance

When grown in the presence of salicylate, *mar* operon deletion mutants of *E. coli* still show increased resistance to multiple antibiotics, but to a much lesser extent than the wild type strain [43]. This suggests, that in *E. coli* at least, other factors are involved with the salicylate-induced multiple antibiotic resistance phenotype. Numerous MarA homologues exist in *E. coli* and other Gram-negative and -positive bacteria, some of which have already been shown to be involved with multiple antibiotic resistance (for review see [45]). The AcrAB efflux pump also has homologues in *E. coli* and other bacteria [56].

The molecular mechanism behind salicylate-induced antibiotic resistance in *S. aureus* is unknown. This organism harbors two intrinsic fluoroquinolone efflux pumps, the multiple drug efflux pump NorA, and another fluoroquinolone efflux pump of unknown identity [57]. It is possible therefore that growth of *S. aureus* in the presence of salicylate stimulates the production of NorA and other as yet unidentified efflux pumps which increase fluoroquinolone and fusidic acid resistance.

The AcrB protein of the *E. coli* AcrAB complex acts as a multidrug efflux pump, while AcrA acts as a conduit to transport drugs through the periplasmic space. These drugs then exit the cell via TolC in the outer membrane [32,33] (Fig. 2). A BLAST search on the uncompleted *S. aureus* genome revealed an open reading frame with strong homology to the *E. coli* AcrB protein (Fig. 4). An additional BLAST search utilizing the putative *S. aureus* AcrB revealed the existence of numerous *S. aureus* AcrB homologues of similar size in many different bacterial genera (Table 2). When aligned, all of the *S. aureus* AcrB homologues showed homology along their entire length as demonstrated by the AcrB consensus sequence generated (Fig. 4). This finding suggests that the AcrB protein is highly conserved in structure and function. It is therefore possible that AcrB is involved with salicylate-induced antibiotic resistance in organisms other than *E. coli*.

Non-growing or slow growing bacteria exhibit increased resistance to antibiotics compared to cells in exponential phase [58]. The transcription factor σ^s encoded by *rpoS*, is expressed under

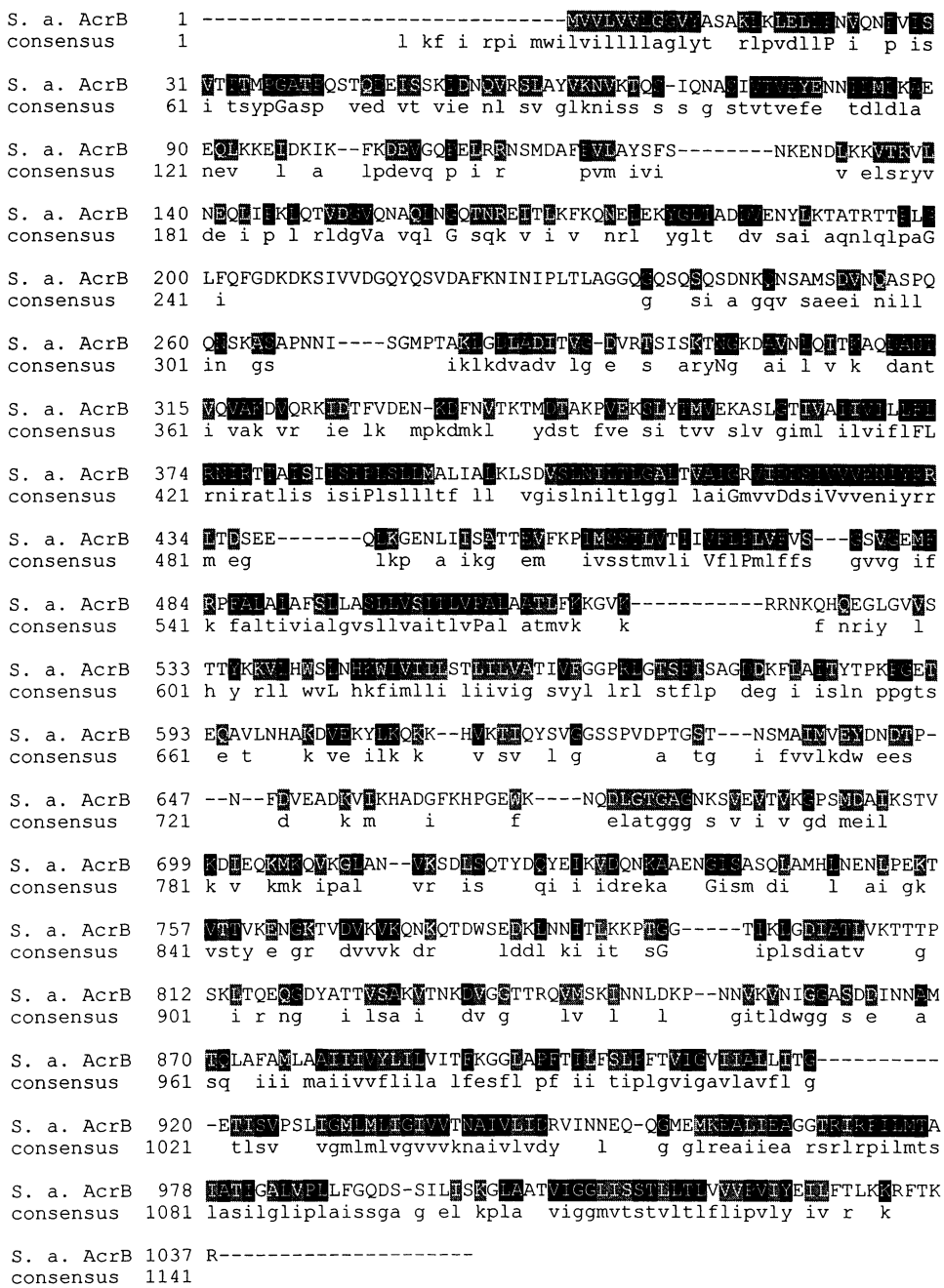

Fig. 4. Alignment of a putative *S. aureus* AcrB homolog (S. a. AcrB) identified from a BLAST search of the uncompleted *S. aureus* genome (http://www.genome.ou.edu/staph.html) with the *E. coli* AcrB, with an AcrB consensus sequence generated from the *S. aureus* AcrB homologues listed in Table 2. In the AcrB sequence, black boxed letters represent amino acids identical in over 50% of the sequences used in the multiple sequence alignment. Grey boxed letters represent amino acid residues identical to the consensus amino acids or residues with similar properties to the consensus amino acids. The multiple alignment and consensus sequence were determined using ClustalW 1.8 and Boxshade 3.21, respectively.

non-growing conditions and directs the production of gene products required for cell survival and maintenance during starvation (for review see [59]) and acid stress [60]. Salicylate, which is a membrane permeant weak acid [61,62] induces the transcription of *rpoS* in *E. coli* and *S. typhimurium* [60,63]. Interestingly, the salicylate inducible multidrug efflux pump *emrKY* is fully expressed during stationary phase [51]. It is therefore possible that the expression of stationary phase genes and/or acid stress genes under the control of σ^s contribute to the increased antibiotic resistance of these organisms when grown in the presence of salicylate.

Salicylate-induced multiple antibiotic resistance is phenotypically expressed. That is, when salicylate is removed from the media, the antibiotic resistance levels return to the pre-induced state [30,41,42]. The antibiotics to which salicylate induces resistance have very different modes of action. Tetracycline, chloramphenicol, thiolactomycin and fusidic acid inhibit protein synthesis. Cephalosporins, carbapenums and β-lactams block peptidoglycan production while nalidixic acid and quinolones inhibit DNA synthesis. It is therefore probable that growth in the presence of salicylate denies access of these drugs to their targets, most of which are located within the bacterial cytoplasm (for review see [29]). Even though the concentration (2 mM) of salicylate required to induce phenotypic antibiotic resistance in bacteria is high, this concentration is used to treat chronic inflammation such as rheumatoid arthritis [64]. It is therefore possible that the efficacy of antibiotic treatment may be impaired in patients taking salicylate and antibiotics simultaneously.

3.3. Salicylate induced antibiotic susceptibility

Salicylate does not induce resistance to all antibiotics, and can actually potentiate the activity of some antibiotics possibly aiding in treatment of some bacterial infections. For example growth of *E. coli* or *K. pneumoniae* in the presence of salicylate increases the antimicrobial activity of aminoglycosides [36,65,66]. It is thought that salicylate enters the cell in a protonated form and then dissociates, thereby increasing membrane potential, which then facilitates entry of these drugs into the cell [67,68]. Treatment efficacy of a *K. pneumoniae*-mouse infection model with the aminoglycoside amikacin is increased by the addition of salicylate in concert [69]. Similarly, efficacy of vancomycin treatment in a *S. aureus*-rabbit endocarditis model is increased with the addition of aspirin to the treatment regimen [70]. Growth in the presence of saligenin, the alcohol of salicylate, has been shown to reduce in vitro resistance to fluoroquinolones and fusidic acid in *S. aureus* [41,42]. The activity of the antifungal fluconazole against the yeast *Candida albicans* is increased by the addition of salicylate or the related compound ibuprofen in vitro [71]. Salicylate and ibuprofen have also been shown to possess antibacterial activity [72–74], and perhaps this activity con-

Table 2
Homologies of bacterial AcrB homologues to *S. aureus* AcrB[a]

Organism	Protein acession no.	Identity (%)	Similarity (%)
Bacillus subtilis	E69795	44.6	64.8
Thermotoga maritima	B72385	24.6	39.6
Aquifex aeolicus	F70368	23.6	41.7
E. coli	P76398	23.5	38.8
Synechocystis sp.	S77008	23.2	37.9
P. aeruginosa	P52002	21.7	38.7
Neisseria meningitidis	CAB85189	20.8	35.2
E. coli	P31224	20.4	34.6
Alcaligenes sp. CT14	P94177	15.0	24.3

[a] Homologies were determined using DNA Strider 1.3.

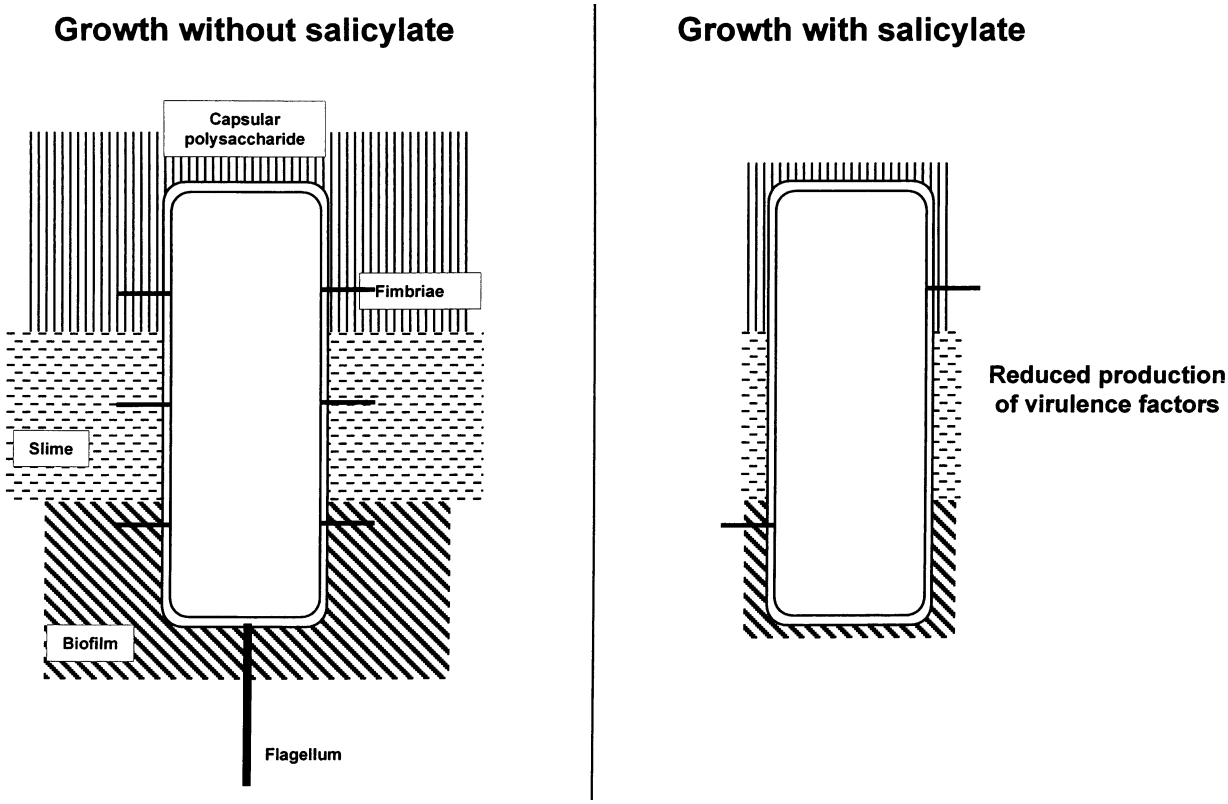

Fig. 5. The effect of salicylate on virulence factor production in a generalized bacterial cell.

tributes to the antimicrobial effect of some antibiotics.

3.4. Effects of salicylate on bacterial virulence factors

Bacterial virulence factors help mediate infection by bacteria in a host organism. Virulence factors are involved with adherence (fimbriae, slime, biofilms), protection against the immune system (capsules, biofilms), susceptibility to antibiotics (biofilms) and iron acquisition. Many reports have indicated that growth in the presence of salicylate affects production of bacterial virulence factors in some bacteria. The overall effect that growth in the presence of salicylate has on virulence factor production in a generalized bacterial cell is shown in Fig. 5.

The synthesis of some types of fimbriae in *E. coli* (colonization factor antigen, P fimbriae and type 1 fimbriae) are reduced following growth in the presence of salicylate [75]. Because fimbriae play a critical role in the attachment of *E. coli* to epithelial surfaces, salicylate treatment might prevent infection caused by some strains of fimbriated *E. coli* [75]. Salicylate also limits adherence of *E. coli* to silastic catheters [76] suggesting that coating catheters with salicylate may prevent *E. coli* infections arising from these prosthetic devices [76]. Growth of encapsulated *K. pneumoniae* in the presence of salicylate results in reduced synthesis of capsular polysaccharides [77,78]. The loss of capsular material exposes the cell surface of *K. pneumoniae* to the host defense mechanisms possibly shortening the time required for infection clearance [79].

Biofilms consist of microorganisms and other matter encased in a polysaccharide matrix of microbial origin [80]. Growth of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* in the

presence of salicylate reduces the production of extracellular polysaccharide required for biofilm formation. This reduction in biofilm formation decreases the ability of these organisms to adhere to contact lenses and medical polymers [81–83]. A component of biofilm production in *S. epidermidis* is extracellular slime which is composed of a complex mixture of polysaccharides, teichoic acids and proteins [84]. Production of slime-associated proteins and teichoic acids is inhibited in *S. epidermidis* by salicylate [85,86]. Coating contact lenses and medical polymers with salicylate may prove to be beneficial in limiting bacterial attachment to these surfaces and hence reduce the occurrence of infection by these organisms [81,82].

P. aeruginosa, *Mycobacterium tuberculosis* and *Burkholderia cepacia* produce the siderophores, pyochelin, mycobactin and salicylate, respectively [87–89]. These siderophores enable these organisms to survive in environments low in iron. In *P. aeruginosa* salicylate is utilized as an intermediate during pyochelin synthesis [87] and the operon, *pchDCBA* is responsible for this process [90,91]. Two proteins, PchA and PchB, encoded by the *pchDCBA* operon are involved in the salicylate biosynthetic pathway in this organism [91]. PchA shares homology with isochorismate synthase, anthranilate synthase and p-aminobenzoate synthase, enzymes which produce substances structurally similar to salicylate [91]. The exact catalytic role of PchB is not known but this protein shares homology to an unknown putative protein in *Vibrio vulnificus* (accession number AAB18149), FbsB from *Pseudomonas fluorescens* (accession number CAA70531) and TyrA from *Haemophilus influenzae* (accession number P43902). FbsB is thought to be involved with salicylate biosynthesis while TyrA is important in tyrosine production. In *M. tuberculosis*, ten genes designated *mbtA* through *mbtJ* are required for mycobactin biogenesis [88]. Of the products produced by the gene cluster, MbtI is an isochorismate synthase required for salicylate biosynthesis [88]. Since the acquisition of iron is important for the survival of organisms during infection [92], it is possible that the inhibition of siderophore production in bacteria may reduce their pathogenicity. A better understanding of the acquisition and production of salicylate might help in the development of therapies against infections caused by these organisms.

Whereas the production of salicylate may be beneficial to the organisms discussed above, salicylate biosynthesis by *P. aeruginosa* 7NSSK2 can be detrimental to bacteria which cause plant disease. It has been shown that the nanogram amounts of salicylate produced by this organism activates SAR in bean plants [93]. The activation of SAR in bean plants by *P. aeruginosa* 7NSSK2 would therefore help prevent plant pathogens from causing disease.

Chemotaxis in bacteria is modulated through regulation of flagella rotation. This rotation when counterclockwise, leads to swimming along a linear trajectory and when clockwise, leads to tumbling [94–96]. Salicylate is recognized as a chemorepellent by the *E. coli tsr* gene product [61]. This recognition leads to prolonged tumbling of motile *E. coli* and ultimately causes cells to migrate away from salicylate [61]. Swarming behavior of *E. coli* is also inhibited by salicylate in a concentration dependent manner [97]. Production of the flagellum itself in *E. coli* is inhibited by growth in the presence salicylate. This is mediated by inhibiting the production of flagellin, the protein monomer constituting the flagella [97]. It has also been speculated that inhibition of flagella synthesis and motility in *E. coli* by salicylate is due to reduced synthesis in OmpF synthesis, which may be required for flagella assembly [97,98]. The swarming activity of *Proteus vulgaris* and *Proteus mirablis* [97] and the motility of *Providencia rettgeri*, *Providencia stuartii* and *B. cepacia* are also reduced when grown in the presence of salicylate [97].

Some bacterial pathogens require a flagella for virulence [99,100]. The sheathed flagella is one virulence factor used by *Helicobacter pylori* to mediate infection of its host [101]. Interestingly, bismuth subsalicylate is used in the treatment of infections caused by this organism, some of which is converted to salicylate at the site of infection [102]. Perhaps the ability of bismuth subsalicylate to clear *H. pylori* infections is in part mediated by reduced motility induced by salicylate. Along these lines, perhaps the production of salicylate by

plants, besides activating SAR, also reduces the motility of flagellated plant pathogens such as *B. cepacia*, thereby undermining the ability of these organisms to cause disease. Thus, the ability of salicylate to inhibit bacterial motility may prove beneficial when it is required as a mediator of infection development.

4. Conclusions

Salicylate has the ability to alter gene expression in many cellular life forms. If one considers this phylogenetically, the only domain which has not yet been shown to be affected by salicylate is the Archaea. This review describes the numerous effects that salicylate has on the bacteria and suggests that salicylate can act as a double-edged sword. On one hand, the drug can reduce the production of various factors bacteria use to mediate infection, act as an antimicrobial and cause a reduction in intrinsic resistance to certain types of antibiotics. On the other hand, salicylate can induce an intrinsic multiple antibiotic resistance mechanism in many different types of bacteria. It must be stated, however, that it is uncertain if this effect diminishes the efficacy of antimicrobial therapies in vivo. Growth in the presence salicylate also increases the mutation frequency by which *S. aureus* becomes resistant to fluoroquinolones and fusidic acid in vitro [41,42]. Fluoroquinolones are broad-spectrum antibiotics use to treat infections caused by numerous bacterial genera [103]. This finding poses the question 'Does the presence of salicyate also induce various bacterial species including *S. aureus* to mutate at a higher frequency to become clinically resistant to fluoroquinolones or other antibiotics in vivo?'. Any increases in the numbers of antibiotic resistant bacteria in the community, or a reduction in the antimicrobial activity of antibiotics is a serious matter, since antibiotic resistant bacteria are already a major public health threat [29].

This review clearly demonstrates that salicylate has a broad effect on the bacterial cell. It also reveals that additional research on the effects of salicylate on bacterial infections is warranted. In particular, the potential decrease or increase in the efficacy of antibiotic therapies by non-steroidal anti-inflammatories in vivo needs to be addressed.

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