BIOINFORMATICS INVESTIGATION OF VIRULENCE FACTORS OF *PROPIONIBACTERIUM ACNES* TARGETED BY EPIGALLOCATECHIN-3-GALLATE (EGCG)

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Abstract

Purpose: This study aimed to investigate and analyze virulence factors of *Propionibacterium acnes* targeted by phytocompound epigallocatechin-3-gallate (EGCG).

Methodology: Several bioinformatics approaches were employed to identify the targets, and evaluate their functional role and virulence property. Additionally, the BepiPred program v.2 was used to identify the peptide epitopes found in the virulence factors. PSORTb v.3 was also used to uncover the subcellular localization of the virulence factors of *P. acnes*.

Findings: EGCG was found to target several virulence factors of *P. acnes* such as Ser/Thr protein kinase, ABC transporter-associated permease, and methylated-DNA--protein-cysteine methyltransferase important for the survival of *P. acnes*.

Limitation: This study was computational research. Therefore, several laboratory experiments are needed to prove this bioinformatics finding.

Contribution: The finding of this study may contribute to the advancement of natural product investigation by uncovering molecular mechanisms of EGCG against *P. acnes*.

Keywords: Bioinformatics, Epigallocatechin-3-gallate (EGCG), Propionibacterium acnes, Virulence factors.

1. Introduction

The largest organ of the body, the skin, is made up of several important microbial groups that are important for skin health. These include Staphylococcus, Propionibacterium, Streptococcus, Corynebacterium, and Malassezia (Barnard & Li, 2017). The Gram-positive anaerobic bacterium *Propionibacterium acnes* is a big part of the normal microbiota of human skin. Furthermore, *P. acnes* is thought to play a key role in the pathophysiology of acne vulgaris, which is a common skin disease (McLaughlin et al., 2019). Additionally, this bacterium has been linked to inflammation and the development of acne (Liu et al., 2015).

Acne vulgaris is the eighth most common disease in the world, affecting about 10% of the world's population (Hay et al., 2014). Symptoms of the disease can range from mild to severe (Moradi Tuchayi et al., 2015). The condition can continue or start for the first time in adulthood, especially for women (Tan et al., 2018). Acne can have serious social and psychological implications, especially when symptoms are severe and scarring occurs (McLaughlin et al., 2019).

The treatment of acne at various ages and in pregnant women is still limited to the use of antibiotics, which might lead to antimicrobial resistance. Antibiotics such as erythromycin and clindamycin can contribute to the development of bacterial resistance in *P. acnes* (Leyden et al., 2011). Therefore, there is a need and a public interest in finding new compounds with better healing properties for acne vulgaris (Hamdy et al., 2017; Sinha et al., 2014). Green tea leaves from *Camellia sinensis* are high in polyphenols like epigallocatechin-3-gallate (EGCG), the main polyphenol in green tea and the most extensively researched (Li et al., 2015). Previous studies reported that EGCG can kill pathogenic bacteria (Jeon et al., 2014; Taylor et al., 2005). Furthermore, EGCG has been documented to inhibit the growth of *Pseudomonas aeruginosa* and *Escherichia coli* isolated from skin wounds (Jeon et al., 2014). Another study showed the antimicrobial activities of EGCG against multi-drug resistance (MDR) *E. coli* and MDR *Staphylococcus aureus* (Parvez et al., 2019). Recently, EGCG has been reported to improve acne in humans by inhibiting *P. acnes* (Yoon et al., 2013). However, the underlying molecular mechanism of EGCG against *P. acnes* has been poorly studied. Therefore, in this study, we aimed to investigate the virulence factors of *P. acnes* targeted by EGCG to elucidate the mechanism of action of EGCG.

2. Research methodology

This study was bioinformatics research involving several computational tools to identify and analyze virulence factors of *P. acnes* targeted by EGCG. The compound protein-interaction prediction was done by STITCH v.5.0. The identified proteins of *P. acnes* targeted by EGCG were then analyzed for their functional class using web-based VICMPred software. The prediction of the virulence property of the proteins was done using the VirulentPred program. In addition, BepiPred v.2 and PSORTb v.3 were employed for epitope and subcellular protein localization analysis, respectively.

3. Results and discussions

Bioinformatics analysis using STITCH v.5.0 identified ten proteins of *P. acnes* targeted by epigallocatechin-3-gallate (EGCG) (Figure 1). Those ten important proteins (upp, PPA0729, PPA1671, PPA1558, PPA0628, PPA1428, PPA0097, PPA1532, PPA0184, and PPA1644) were shown in identifier codes with different protein names, function, and property. Interestingly, some proteins targeted by EGCG had interacted with one another (PPA1644, upp, PPA0729, and PPA0184).
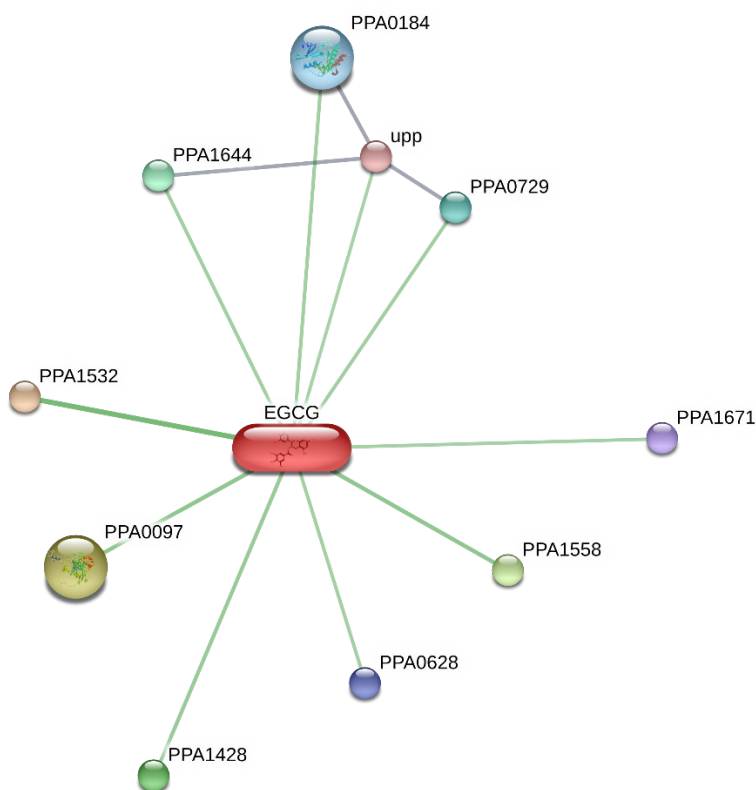

Figure 1. Interaction of EGCG with the significant proteins of *P. acnes*

EGCG was found to interact with a plethora of crucial proteins in *P. acnes* (Table 1). Those crucial proteins were related to cellular processes including uracil phosphoribosyltransferase, dihydrofolate

reductase, N-succinyldiaminopimelate aminotransferase, methylated-DNA--protein-cysteine methyltransferase and serine/threonine protein kinase (PPA1644). Meanwhile, Ser/Thr protein kinase (PPA0729), ABC transporter-associated permease, catalase, Ser/Thr protein kinase (PPA0184), and enoyl-ACP reductase were associated with the metabolism of *P. acnes*. Interestingly, in this study, we highlighted that serine/threonine protein kinase (PPA1644), Ser/Thr protein kinase (PPA0729), and Ser/Thr protein kinase (PPA0184) were the same type of kinase, however, VICMPred and VirulentPed analysis identified they were different in terms of functional class and virulent property. Further analysis among proteins of *P. acnes* using VirulentPred identified four proteins considered as virulence factors of *P. acnes*, namely Ser/Thr protein kinase (PPA0729), ABC transporter-associated permease, methylated-DNA--protein-cysteine methyltransferase, and serine/threonine protein kinase (PPA1644) with VirulentPred Score of 0.847, 1.054, 0.6193, and 0.8824, respectively.

Table 1. List of proteins of *P. acnes* that interacts with EGCG

Organism	Identifier	Protein which interacts with EGCG	VICMPred Functional Class	VirulentPred	VirulentPred Score
P. acnes	upp	uracil phosphoribosyltransferase	Cellular process	Non-Virulent	-0.302
	PPA0729	Ser/Thr protein kinase	Metabolism	Virulent	0.847
	PPA1671	dihydrofolate reductase	Cellular process	Non-Virulent	-1.022
	PPA1558	ABC transporter-associated permease	Metabolism	Virulent	1.054
	PPA0628	N-succinyldiaminopimelate aminotransferase	Cellular process	Non-Virulent	-0.913
	PPA1428	methylated-DNA--protein-cysteine methyltransferase	Cellular process	Virulent	0.6193
	PPA0097	catalase	Metabolism	Non-Virulent	-0.865
	PPA1532	enoyl-ACP reductase	Metabolism	Non-Virulent	-0.128

PPA1644	serine/threonine protein kinase	Cellular process	Virulent	0.8824
PPA0184	Ser/Thr protein kinase	Metabolism	Non-Virulent	-0.944

The four important virulent proteins of *P. acnes* were then predicted their epitope sites using Immune Epitope Database (IEDB). The results of epitope prediction showed that Ser/Thr protein kinase (PPA0729), ABC transporter-associated permease (PPA1558), methylated-DNA--protein-cysteine methyltransferase (PPA1428), and serine/threonine protein kinase (PPA1644) possessed 13, 9, 8, and 13 sites of the epitope, respectively (Figure 2). The identification of many peptide epitopes justified the use of EGCG as the phytocompound with antimicrobial activity against *P. acnes*.
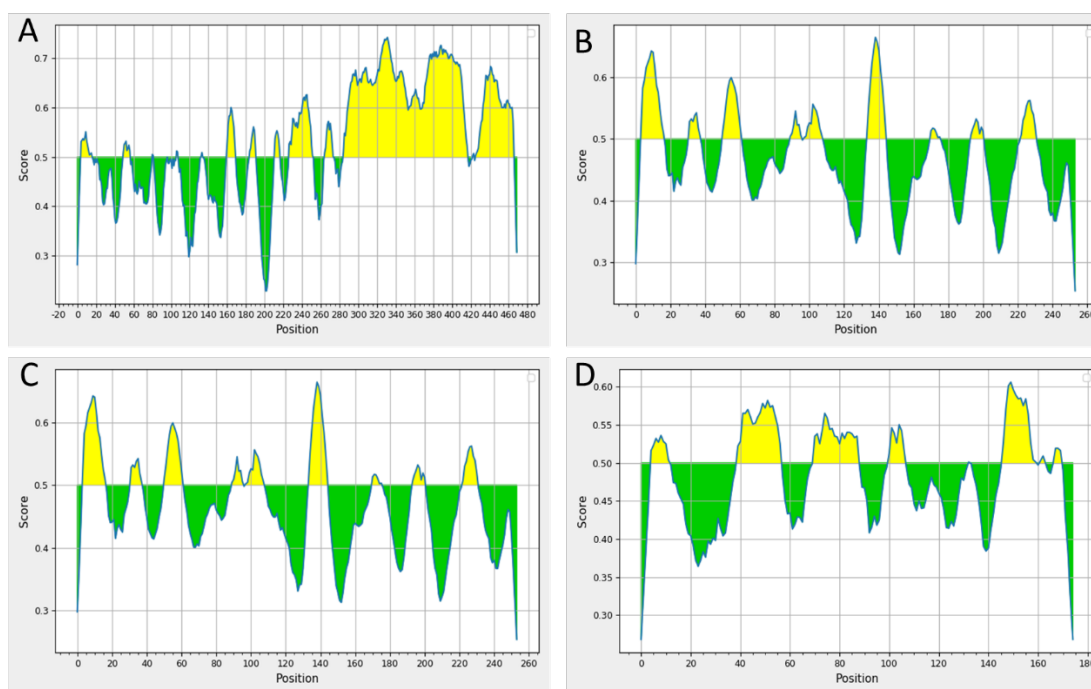

Figure 2. Predicted epitopes on the virulence protein (A) Ser/Thr protein kinase (PPA0729), (B) ABC transporter-associated permease (PPA1558), (C) methylated-DNA--protein-cysteine methyltransferase (PPA1428), and (D) serine/threonine protein kinase (PPA1644).

Furthermore, the subcellular localization of each virulent protein was studied by using PSORTb v.3. It has been identified that Ser/Thr protein kinase (PPA0729) and ABC transporter-associated permease (PPA1558) were localized in the cytoplasmic membrane. Meanwhile, the localization of

methylated-DNA--protein-cysteine methyltransferase (PPA1428) was unknown and serine/threonine protein kinase (PPA1644) was identified in cytoplasmic (Table 2).

Table 2. Subcellular localization of virulent proteins of *P. acnes*

Organism	Protein identifier	Functional protein	Subcellular location
P. acnes	PPA0729	Ser/Thr protein kinase	Cytoplasmic Membrane
	PPA1558	ABC transporter-associated permease	Cytoplasmic Membrane
	PPA1428	methylated-DNA--protein-cysteine methyltransferase	Unknown
	PPA1644	serine/threonine protein kinase	Cytoplasmic

In the previous study, EGCG decreased the viability of *P. acnes* and most importantly, EGCG significantly improved acne in an 8-week randomized, split-face, clinical trial, and was well tolerated (Yoon et al., 2013). The mechanism of actions of EGCG against *P. acnes* has been studied in this research. Our computational study found four virulence factors of *P. acnes* targeted by EGCG including Ser/Thr protein kinase (PPA0729), ABC transporter-associated permease (PPA1558), methylated-DNA--protein-cysteine methyltransferase (PPA1428), and serine/threonine protein kinase (PPA1644). Those four important proteins were critical for *P. acnes* since they were associated with metabolism and cellular process. Specifically, the virulence determinants of pathogenic bacteria consist of sensor/signaling proteins of the serine/threonine protein kinase (STPK) family, which have the dual function of sensing the environment and subverting specific host defensive systems. STPKs can detect a wide variety of signals and coordinate many cellular processes to generate an appropriate response. STPKs are crucial virulence factors that modulate global host responses during infection (Canova & Molle, 2014). ABC transporters serve either as importers, carrying nutrients and other molecules into cells, or as exporters, pumping toxins, drugs, and lipids across cell membranes (Rees et al., 2009). In addition, ABC transporters play a crucial role in bacterial pathogenesis and virulence as well as facilitate bacterial pathogenicity by facilitating the acquisition of essential nutrients (Akhtar & Turner, 2022). In the meantime, methylated-DNA-protein-cysteine methyltransferase (MGMT) adopts a secondary structure of a three-stranded antiparallel β-sheet and three alpha helices. It has been hypothesized that MGMT confers

thermostability to the protein, however, its precise role remains unknown (Hashimoto et al., 1999). The interactions between EGCG and proteins of *P. acnes* that have been identified in this study may answer how EGCG works against *P. acnes*. However, several experiments are important to be conducted to confirm our findings.

4. Conclusion

Epigallocatechin-3-gallate (EGCG) can be employed as an antimicrobial agent to eradicate *P. acnes*. The mode of action of EGCG as an inhibitor of Ser/Thr protein kinase, ABC transporter-associated permease, and methylated-DNA--protein-cysteine methyltransferase will make it an ideal medication for use in therapeutic applications.

Limitation and study forward

This research was a bioinformatics study. Therefore, the findings of this study can be confirmed by the laboratory experiments to prove the real molecular mechanism of EGCG against *P. acnes*.

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