

ECO-FRIENDLY BACTERIAL PRODIGIOSIN-BASED ANTIBIOFOULING
COATING FOR MARINE STRUCTURE APPLICATIONS

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ABSTRACT

Marine biofouling, the unwanted accumulation of living organisms on manmade structures is worsening and creating an adverse economic impact. In this study, a more sustainable antibiofouling paint was formulated by using natural antibiofouling agent. Prodigiosin, a bacterial pigment extracted from *Serratia marcescens* was used as the antibiofouling agent and pigment. The antibiofouling properties of crude prodigiosin were studied by assessing the antibacterial properties, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and toxicity assay. The performance of antibiofouling paint at different pigment composition incorporated with crude prodigiosin was studied in laboratory and in natural seawater. Based on the result obtained, the antibacterial test demonstrated the largest inhibition zone against *B. subtilis* (4.0 ± 0.3 cm) at 10000 ppm and the smallest inhibition zone against *E. coli* (2.0 ± 0.4 cm) at 1000 ppm. The MIC values ranged from 390 to 3126 ppm and MBC values ranged from 1562 ppm to 6250 ppm for all bacteria tested. Crude prodigiosin showed a low toxicity value (LC50 = 4270.203 ppm). The comparative study of binder showed that vinyl ester resin performed better in comparison to epoxy resin in all tests, namely antibacterial test (*B. subtilis*: 3.77 ± 0.1 cm, *E. coli*: 3.43 ± 0.1 , *P. aeruginosa*: 3.63 ± 0.3 , *S. aureus*: 4.00 ± 0.1), surface roughness and adhesion test (Class 5B). Steel plate painted with crude prodigiosin was able to retain its cleanliness at high flowrate areas (significant, $p \leq 0.05$) in natural seawater while a thinner mud settlement was observed at low flowrate areas (not significant, $p \geq 0.05$). Based on the research outcomes, it was observed that with thorough study, prodigiosin has a high potential as an effective environmental friendly antibiofouling agent since it can inhibit the attachment of marine organisms on wetted surfaces.

ABSTRAK

Biokotoran laut, iaitu pengumpulan organisma hidup yang tidak diinginkan pada struktur buatan manusia kian semakin teruk dan memberi kesan ekonomi yang buruk. Dalam kajian ini, cat antibiokotoran yang lebih mampan telah dirumus dengan menggunakan ejen antibiokotoran semula jadi. Prodigiosin, pigmen bakteria yang diekstrak daripada *Serratia marcescens* telah digunakan sebagai agen antibiokotoran dan pigmen. Ciri-ciri antibiokotoran prodigiosin mentah telah dikaji dengan menilai sifat antibakteria, kepekatan perencatan minimum (MIC), kepekatan bakteria minimum (MBC) dan pengujian ketoksikan. Prestasi cat antibiokotoran pada komposisi pigmen yang berbeza digabungkan dengan prodigiosin mentah telah dikaji di makmal dan di dalam air laut semula jadi. Berdasarkan keputusan yang diperolehi, ujian antibakteria menunjukkan zon perencatan terbesar terhadap *B. subtilis* (4.0 ± 0.3 cm) pada 10000 ppm dan zon perencatan terkecil terhadap *E. coli* (2.0 ± 0.4 cm) pada 1000 ppm. Nilai MIC adalah antara 390 hingga 3126 ppm dan nilai MBC adalah antara 1562 ppm hingga 6250 ppm bagi semua bakteria yang diuji. Prodigiosin mentah menunjukkan nilai ketoksikan yang rendah ($LC_{50} = 4270.203$ ppm). Kajian perbandingan perekat menunjukkan bahawa resin vinil ester menunjukkan prestasi yang lebih baik berbanding dengan resin epoksi dalam semua ujian, iaitu ujian antibakteria (*B. subtilis*: 3.77 ± 0.1 cm, *E. coli*: 3.43 ± 0.1 , *P. aeruginosa*: 3.63 ± 0.3 , *S. aureus*: 4.00 ± 0.1), kekasaran permukaan dan ujian perekatan (Kelas 5B). Keluli yang dicat dengan prodigiosin mentah dapat mengekalkan kebersihannya di kawasan kadar alir yang tinggi (signifikan, $p \leq 0.05$) di dalam air laut semula jadi sementara mendapan lumpur yang lebih nipis telah diperhatikan di kawasan kadar alir yang rendah (tidak signifikan, $p \geq 0.05$). Berdasarkan hasil penyelidikan, diperhatikan bahawa dengan kajian menyeluruh, prodigiosin mempunyai potensi yang tinggi sebagai ejen antibiokotoran mesra alam yang berkesan kerana ia boleh menghalang organisma laut daripada melekat di atas permukaan yang dibasahi.

DECLARATION	iii
DEDICATION	iv
ACKNOWLEDGEMENT	v
ABSTRACT	vi
ABSTRAK	vii
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF EQUATIONS	xvi
LIST OF ABBREVIATIONS	xvii
LIST OF SYMBOLS	xviii
CHAPTER 1 INTRODUCTION	1
1.1 Background of Study	1
1.2 Problem Statement	3
1.3 Research Objectives	4
1.4 Scopes of Study	5
1.5 Significance of Study	6
CHAPTER 2 LITERATURE REVIEW	7
2.1 Marine Fouling	7
2.2 Mechanism of Biofouling	8
2.3 Main Biofouling Organisms	9
2.3.1 Microfoulers	10
2.3.1.1 Microalgae	10
2.3.1.2 Bacteria	11
2.3.1.3 Fungus	12
2.3.2 Macrofoulers	13
2.3.2.1 Barnacles	13
2.3.2.2 Seaweeds	14
2.3.2.3 Mussels	15
2.3.2.4 Byozoans	16
2.3.2.5 Polychaetes	17
2.4 Factors Affecting Biofouling	18

2.4.1	Salinity of Water Body	19
2.4.2	Temperature	19
2.4.3	Nutrient Level	20
2.4.4	pH	21
2.4.5	Flow Rates	21
2.4.6	Surface Topography	21
2.4.7	Duration and Intensity of Light	22
2.5	Damage Caused by Biofouling	23
2.5.1	Functional, Structural and Economic Impact	23
2.5.2	Environmental Impact	25
2.5.3	Health and Environmental Impact	27
2.6	Biological Approach for Biofouling Problem	28
2.6.1	Prodigiosin as Antibiofouling Agent	30
2.7	Composition of Antibiofouling Paint	31
2.7.1	Binder and Plasticizer	32
2.7.2	Solvent	33
2.7.3	Pigment	33
2.8	Critical Test for Antibiofouling Paint	34
2.8.1	Physical Properties of Paint	34
2.8.2	Accelerated Weathering	34
2.8.3	Field Immersion Test	35
CHAPTER 3	METHODOLOGY	37
3.1	Preparation of Glassware	37
3.2	Preparation of Peptone	37
3.3	Preparation of Nutrient Broth	38
3.4	Preparation of Nutrient Agar	38
3.5	Preparation of Artificial Seawater for <i>Artemia salina</i>	39
3.6	Hatching of Brine Shrimp (<i>Artemia salina</i>)	40
3.7	Preparation of Bacteria Culture	40
3.8	Extraction of Prodigiosin from <i>Serratia marcesens</i>	41
3.9	Screening for Antibiofouling Properties	41

3.9.1	Antibacterial Assay	41
3.9.2	Minimum Inhibitory Concentration (MIC)	43
3.9.3	Minimum Bactericidal Concentration (MBC)	43
3.9.4	Toxicity Test on <i>Artemia salina</i>	44
3.9.5	Field Immersion Test of Polymer Tubes	44
3.10	Formulation of Paint	48
3.10.1	Formulation of Paint using Different Types of Binder and Plasticizer	48
3.10.2	Formulation of Paint using Various Ratio of Pigment	50
3.10.3	Formulation of Paint for Field Immersion Test	50
3.11	Critical Testing of Paint	51
3.11.1	Adhesion Test	51
3.11.2	Light Fastness Test	54
3.11.3	Field Immersion Test	54
3.11.4	Dry Weight	56
3.12	Research Flowchart	57
CHAPTER 4	RESULT AND DISCUSSION	59
4.1	Extraction of Crude Prodigiosin from <i>Serratia marcescens</i>	59
4.1.1	Cultivation of <i>Serratia marcescens</i> on Nutrient Agar plate	59
4.2	Preliminary Study of Crude Prodigiosin as Antibiofouling Agent	61
4.2.1	Antibacterial Activity of Crude Prodigiosin	61
4.2.2	Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)	64
4.2.3	Toxicity Test on <i>Artemia salina</i>	65
4.2.4	Field Immersion Test of Crude Prodigiosin on Polymer Tubes	67
4.3	Comparative Study Between Two Types of Binder and Plasticizer	70
4.3.1	Antibacterial Activity on Different Binders	71
4.3.2	Adhesion Test by Tape Method	74

4.3.3	Surface Roughness	78
4.3.4	Light Fastness	82
4.4	Crude Prodigiosin as Antibiofouling Agent in Paint Formulation	83
4.4.1	Effect of Crude Prodigiosin Concentration	83
4.4.1.1	Antibacterial Activity	83
4.4.1.2	Light Fastness Test	86
4.4.1.3	Adhesion Test	87
4.4.1.4	Surface Roughness	88
4.4.2	Field Immersion Test	89
4.4.2.1	Performance of Antibiofouling Paint	90
CHAPTER 5	CONCLUSION AND RECOMMENDATION	95
5.1	Conclusion	95
5.2	Recommendations	96
	REFERENCES	97
	LIST OF PUBLICATIONS	117

LIST OF TABLES

TABLE NO.	TITLE	PAGE
Table 2.1	Summary of antibiofouling method	29
Table 2.2	Composition of antibiofouling paint	31
Table 2.3	Some binder used in antibiofouling paint	32
Table 3.1	Composition of Instant Ocean sea salt mixture	39
Table 3.2	Dilution table of crude prodigiosin for antibacterial test	42
Table 3.3	Sample description for field immersion test of polymer tubes	45
Table 3.3	Sample description for field immersion test of polymer tubes (continuation)	46
Table 3.4	Condition of seawater before field static immersion test for polymer tubes	48
Table 3.5	Composition of antibiofouling paint for comparative study of binder on acrylic plate	49
Table 3.6	Working volume of antibiofouling paint for comparative study of binder on acrylic plate	49
Table 3.7	Formulation of paint using different pigment composition	50
Table 3.8	Composition of antibiofouling paint for field immersion test on mild steel plate	51
Table 3.9	ASTM classification for adhesion test by using tape method	52
Table 3.9	ASTM classification for adhesion test by using tape method (continuation)	53
Table 3.10	Condition of seawater before field static immersion test	55
Table 3.11	Painted mild steel plate for field static immersion test	56
Table 4.1	Antibacterial activities of crude prodigiosin diluted in dimethyl sulfoxide	62
Table 4.2	MIC and MBC value for crude prodigiosin	64
Table 4.3	Toxicity test of crude prodigiosin on <i>Artemia salina</i> (LC ₅₀ = 4270.403 ppm)	66
Table 4.4	Initial and final condition of tube for field immersion test	68

Table 4.5	Observation on the tube after field immersion test	69
Table 4.6	Inhibition zone of different binder composition	72
Table 4.7	Paint formulation for different types of binder	75
Table 4.8	Adhesion Rate of Different Binder	76
Table 4.9	Surface roughness of different binder	78
Table 4.9	Surface roughness of different binder (continuation)	79
Table 4.10	Antibacterial test of different pigment composition	84
Table 4.11	Adhesion test for different pigment composition on acrylic plate	87
Table 4.12	Surface roughness of painted acrylic plate	88
Table 4.13	Composition of antibiofouling paint for field immersion test	89
Table 4.14	Condition of steel plate before and after field immersion test	91
Table 4.15	Effect presence of crude prodigiosin in paint formulation on biofouling attachment on mild steel plate	93

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
Figure 2.1	Mechanism of biofouling formation	9
Figure 2.2	Microalgae under light microscope (1000x)	11
Figure 2.3	<i>Aeromonas</i> sp. on red blood agar (left) and <i>Pseudomonas</i> sp. on nutrient agar (right)	12
Figure 2.4	<i>Penicillium</i> sp. on nutrient agar	13
Figure 2.5	Barnacles (left), barnacles on boat (right)	14
Figure 2.6	Seaweed (left), seaweed fouling on boat (right)	15
Figure 2.7	Mussel (left), mussels fouling in pipeline (right)	16
Figure 2.8	Byrozoan (left), bryozoan fouling on fish net (right)	17
Figure 2.9	Polychaetes (left), Polychaetes fouling on rocks (right)	18
Figure 2.10	Biofouling underwater on oil platform structure	25
Figure 3.1	Disc arrangement of antibacterial test	42
Figure 3.2	Location for polymer tube field immersion test [blue mark (location 1), white mark (location 2), green mark (location 3)]	47
Figure 3.4	Location for static immersion test [red mark (location 4), yellow mark (location 5)]	55
Figure 4.1a	<i>Serratia marcescens</i> growing on nutrient agar plate	59
Figure 4.4	Toxicity test of crude prodigiosin against <i>A. salina</i>	66
Figure 4.6	Antibacterial activity of paint formulated by using two different types of binders	73
Figure 4.7	Condition of adhesion test on acrylic plates	75
Figure 4.8	Condition of paint after immersion in water at 40 °C for 2 weeks	80
Figure 4.9	Resin lost to the water in vinyl ester resin (left) and epoxy resin (right)	81
Figure 4.10	Delta E of formulated paint	82
Figure 4.11	Antibacterial activity of different crude prodigiosin composition	85

Figure 4.12 Light fastness of different pigment composition on acrylic plate

86

LIST OF EQUATIONS

- 1.1 Inhibition zone for antibacterial activity
- 1.2 Mass of prodigiosin infused in polymer tubes
- 1.3 Delta E value for paint formulation

LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Materials
CFU	Colony forming unit
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ER	Epoxy resin
ERO	Epoxy resin with oil
ERS	Epoxy resin with shellac
HCl	Hydrochloric acid
NA	Nutrient agar
NaOH	Sodium hydroxide
NB	Nutrient broth
VOC	Volatile Organic Compound
MEKP	Methyl ethyl ketone peroxide
µg	Microgram
mg	Miligram
mL	Mililiter
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
nm	Nanometer
NA	Nutrient agar
NB	Nutrient broth
UV	Ultraviolet
VE	Vinyl ester resin
VEO	Vinyl ester resin with oil
VES	Vinyl ester resin with shellac

LIST OF SYMBOLS

°C	Temperature relative to 273.15 K
cm	Length
mm	Length
g	Mass
g/L	Mass concentration
rpm	Revolutions per minute
kPa	Pressure
L	Volume
μL	Volume
M	Molarity
mg	Mass
mg/m ³	Concentration
ms ⁻¹	Velocity
ppm	Volume (mg/L)

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Marine biofouling, the unwanted accumulation of living organisms on man-made structures is worsening every year (Vinagre *et al.*, 2020). They grow on structures including deep sea oil platform, pipelines, fish nets, ships, cables as well as the bridge pillars. They basically grow on anything submerged in the seawater including on the living things such as crab and turtles. Biofouling in marine environment usually occurs in any wetted surface, submerged for a period of time in the seawater. This phenomenon happens in few stages. Marine biofouling started with conditioning followed by adhesion and attachment of microorganisms and consequently the attachment of larger organisms or macroorganisms. Conditioning process produces adhesins which lead to the next stage, adhesion of microorganisms (Li *et al.*, 2016). Microorganisms on the other hand produces biofilm which facilitate the attachment of larger organisms on the affected surface. The whole process of formation of biofouling occurs within a week. Bacteria species that usually form biofilm in marine environment are *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Eschericia coli* (Magana *et al.*, 2018; Jamal *et al.*, 2018; Schinke *et al.*, 2017).

Marine biofouling is a problematic issue that needs immediate action. This is due to the significant impact of marine biofouling on the daily operation of most sectors associated with marine environment. Some of serious problems include the significant increase in expenses for fuel due to an increase in drag resistance and weight, cleaning the formed biofouling, harmful waste scraped from fouled surfaces into the sea, profit loss for dry docking and reduction in shelf life for the man-made structure (Wang *et al.*, 2018; Hakim *et al.*, 2019). Biofouling affects the aquaculture environment, shipping and power industries and water purification (Bannister *et al.*, 2019; Forrest & Atalah, 2017).

The basic composition of the antibiofouling paint comprises of binder, solvent, antibiofouling agent, additives and pigment (Watermann & Eklund, 2019). Binder acts as the agent that hold all components together on the surface of interest. In order for the paint to be possible to be applied on surface, vehicle is needed. Solvent acts as the vehicle for the paint to be applied on the surface of marine structure which could either be organic solvents or water. In the case of antibiofouling paint, organic solvent is preferred over water as to produce a non-water based paints. Additives are optional as they are added to improve the performance of the paint. Some of the common additives used in antibiofouling paints are the pH regulator and anti-corrosive additives (Fay *et al.*, 2019).

The International Maritime Organization (IMO) has strictly outlined the standards that needs to be complied by the antibiofouling paint manufacturers as to defend the fate of mother earth. Thus, to develop next of a more sustainable and environmental friendly paint for marine structure applications is needed. Consequently, biofouling is a significant management issue resulting in increased operational expenses. The commonly available in local market antibiofouling paints are Lalizas (Greece), Jotun (Norway) and Nippon (Japan). Hence, a greener and effective approach is highly on demand as to combat this marine biofouling problem as well as to protect the mother earth especially the fate of marine life (Zhou *et al.*, 2009).

Up to this day, there is still no promising solution for this biofouling problem. With regard to environmental concern, biofouling makes the man-made structure to not be able to function in its maximum potential within a very short period of time. In the early development of antibiofouling paint, copper is used to prevent biofouling on ship hulls. Problem arises as the copper incorporated in the antibiofouling paint could only last for a very short period of time due to no control release of the copper causing rapid leaching of the antibiofouling agent (Xie *et al.*, 2013). Besides, this method is also observed to significantly increase the accumulation of copper salts in the marine environment. After that, biocides were used as an antibiofouling agent (Zhang *et al.*, 2000).

Serratia marcescens (*S. marcescens*), a gram negative rod-shaped bacteria that occurs naturally in water and soil (Akl *et al.*, 2020). *S. marcescens* produces the red pigment prodigiosin under desired condition. Prodigiosin (C₂₀H₂₅N₃O) is a secondary metabolite with a wide range of medicinal and industrial benefits (Lapenda *et al.*, 2020). Prodigiosin has demonstrated its potential in various applications as the food and polyolefines colorant, sunscreen, antimalarial, antibacterial and anticancerous agent (Khanam & Chandra, 2018). The antibiofouling paints made from bacterial pigment will result in a non-hazardous scrap waste since it is originated from the environment.

1.2 Problem Statement

It is apparent that biological materials have a higher tendency to attach and settle on a super-hydrophobic surface, the surface modification method has also been developed just to bring about only a little success in terms of efficiency and service life. Currently, only limited products are available to control biofouling in marine structures (Gooch & Hlady, 2015). Biocides have been proven to be an effective antibiofouling agent for up to 5 years (Joshi *et al.*, 2015). Despite its effectiveness, the environmental consequences are concerning too due to its high level of toxicity against marine organisms. A self-polishing paint can last long for up to 4 years but however contained a biologically toxic organotin compound such as tributyltin oxide and remarkably toxic to marine organisms at a very low concentration. However, all these methods have their own limitations (Zhou *et al.*, 2009).

Current method used against the biofouling problem is not environmental friendly. The antibiofouling paint currently used usually contains heavy metals, continuously leached out killing microfoulers to prevent the attachment of microfoulers as to protect the surface against the biofouling organisms (Lagerstrom *et al.*, 2020). Hence, a safer, environmental-friendly and last long method is in need to combat this problem (Zhang *et al.*, 2000).

In facts that the current worn out antibiofouling paints need a mechanical action to be removed so that the new antibiofouling paints can be applied, the scraped waste usually goes in back to the sea (Muller *et al.*, 2021). The unutilized biofouling agents that goes to the sea will continue poisoning marine life, which is the non-target organisms (Matin *et al.*, 2011). Bacterial pigment is chosen to substitute the currently used antibiofouling agents for several reasons. One of the reason is mainly because of its abundance in the world. Bacteria is easily available all around us, in abundance amount. It is easy to get the bacteria of interest by isolation method. In addition, bacteria have a short life cycle and can be easily grown in a large scale in a short period of time. In compared to the current antibiofouling paints that not only prevent the biofouling problem but also highly toxic to the life in its area, bacterial pigment will act as the biofouling agent by killing target organism, not all organisms living in the area.

1.3 Research Objectives

This study will access a new antibiofouling paint by using prodigiosin as the antibiofouling agent. Currently, the antibiofouling paint used in the marine industry is not environmental friendly. A safe way to solve this problem is by using the natural antibiofouling agent. Therefore, in this study, it is proposed the use prodigiosin due to its high antibacterial properties. Since the biofouling problem started with settlement of bacteria on immersed surface, the antibacterial properties of prodigiosin has the potential to prevent the settlement of bacteria. The objectives of the research are:

- (a) To determine the antibiofouling properties of the prodigiosin.
- (b) To formulate the antibiofouling paint comprising of various composition of prodigiosin.
- (c) To evaluate the performance and physical characteristics of the antibiofouling paint in seawater.

1.4 Scopes of Study

To achieve all objectives stated in 1.3, a few experimental procedures was carried out according to the objectives stated. To fulfil the first objective, the study first began by screening for the antibiofouling properties of a bacterial pigment, the antibacterial test on marine bacteria namely *Bacillus subtilis*, *Eschericia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* using the disc diffusion method was conducted. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the pigment produced from bacteria against the marine bacteria was conducted as well. This is to determine the concentration at which the crude prodigiosin is able to inhibit the bacterial growth and hence, preventing primary attachment of bacteria on wetted surface in marine structure. Next, to evaluate the toxicity of pigment extracted from bacteria against marine organism namely *Artemia salina*, the in-vivo toxicity test of pigment against brine shrimp was conducted as to determine the toxicity level of the pigment against marine life and human. This is important as to achieve the Sustainable Development Goals, goal number 9 the life below water (Zhuiykov, 2014).

The second objective is to formulate the antibiofouling paint using the crude prodigiosin extracted from *Serratia marcesens*. A number of different formulations with varying ratio crude prodigiosin was tested and monitored for efficacy to prevent biofouling using adhesion test and shelf life test on acrylic plates for up to 14 days.

The third objective is to evaluate the performance of antibiofouling paint formulation and perform a physical characterization on the antibiofouling paint painted on mild steel plate which represent the ship hull for 8 weeks. To achieve this objective, field immersion test in natural seawater was conducted. The dry mass was determined. The antifouling performance was evaluated by evaluating the colour change, dry mass of coated mild steel plates and observation on surface coated after immersion test was conducted.

1.5 Significance of Study

The development of an efficient and environmental friendly antibiofouling paint is crucial. Hence, by evaluating the antibiofouling properties of potential compounds is a promising action in order to produce efficient antibiofouling paint to replace the existing harmful antibiofouling paint. Most people are still unconcerned on the fate of the environment on the currently used antibiofouling paints (Song *et al.*, 2020).

Marine life is extremely suffering due to the existing antibiofouling paint, and human and other living organisms will face the same fate if the problem is continuously left unaddressed. Production cost is one of the major problem in developing a sustainable antibiofouling paints. As for bacterial pigment, because of its abundance in the environment and can be easily grown in a large scale in a short period of time, it is also can be grown in a cheap growth medium from waste industrial materials. Hence, it contributed to a lower production cost and reducing waste from industries to be utilized in a good mean (Farkas *et al.*, 2020).

In addition, a greener technology leads to a greener environment by reducing the fuel consumption for operation by eliminating the biofouling problem as less greenhouse gasses will be produced by burning of fossil fuels too. Bacterial based antibiofouling paint is a promising candidate to produce an antibiofouling paint as efficient as the currently available in market, but less toxic (Gallagher *et al.*, 2015).

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