ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.9, No.01 pp 208-217, 2016

Marine organisms: A potential source of natural antifouling metabolites

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Abstract: Marine organisms have been proven to be excellent sources of environmentally benign biogenic compounds. These metabolites have received much attention because of their pharmacological and antifouling activites. Biofouling process is known to cause serious problems to the submerged structures. Many countries have ratified an international treaty to ban the application of antifouling coatings based on organotin compounds. This has underlined the urgent demand for environmentally benign, non-toxic antifouling compounds to substitute the efficient but extremely toxic antifouling. Members of marine macroorganisms which include sponges, sea weeds, sea anemone etc. and microorganisms viz. bacteria, fungi, and actinomycetes have been reported to produce antifouling compounds. This review attempts to bring together the bioprospection for natural antifouling compounds which would be potential candidates in the development of environmentally benign antifouling paints.

Key words: Biofouling, antifouling, larval settlement, biofilm, microorganism.

Introduction

'Biofouling' is the colonisation of submerged surfaces by unwanted organisms such as bacteria, barnacles and algae. It has detrimental effects on shipping and leisure vessels, heat exchangers, oceanographic sensors and aquaculture systems. High frictional resistance, caused by the development of fouling on hulls of ships leads to an increase of weight and subsequent potential speed reduction and loss of maneuverability. To compensate for this, higher fuel consumption is needed, which causes increased emissions of harmful compounds. A vessel with a fouled hull burns 40% more fuel which has an impact on fuel costs and additional greenhouse gas production. In shipping industry, the use of antifouling coatings could save around 20 billion Euros per year. Fouled hulls are also implicated in the spread of 'alien species' around the world, potentially threatening the balance of sensitive ecosystems.

The influence of biofouling on coastal and oceanographic measuring instruments, which are routinely used in marine and coastal research and monitoring programmes, is very strong. In the early stages of biofoulingie, within a few days of immersion, there is a significant impact on data quality and instrument performance. There is a need to protect the instruments from biofouling so that they are able to gather better quality data and require less maintenance. Currently there are no effective coatings to control this problem; the only solution involves expensive manual cleaning by divers.

In desalination and power plants biofouling of intake structures, screens, seawater piping systems and heat-exchanger tubes causes an overall decline in plant efficiency at great economic cost. For example the presence of a biofilm on transfer surfaces of heat exchangers cooled by seawater reduces the heat transfer rate by 20 to 50% and incurs a global expenditure of over $15 billions per annum to control the problem. The majority of current measures to control biofouling involve the use of biocides. Since man's first venture into the sea strategies for containing fouling have probably been initiated. Broad-spectrum metal biocides, such as tributyl tin (TBT) and copper, have been added to marine paints as antifouling compounds [1,2]. Although very effective, these biocides are often extremely toxic to a wide range of non-target organisms[3,4]. For example, gastropod imposex, mussel larvae mortality, and oysteFr shell malformation have all been recorded as ecotoxicological effects of TBT even at extremely low concentrations (in the ng/L range)[5]. The increasing concerns about the negative effects of TBT led the International Maritime Organization (IMO) and Marine Environmental Protection Committee (MEPC) to decide to ban the usage of TBT or other substances containing tin as biocides in antifouling paints beginning in January 2008. An effective alternative to TBT is not currently available; therefore, there is an urgent demand for the development of environment- and human-friendly non-toxic antifouling compounds.

The restrictions imposed on the use of heavy-metal based antifouling coatings has prompted the search for more environmentally benign antifoulants, and one of the most promising alternative technologies is the use of naturally occurring antifouling compounds derived from natural metabolites from marine organisms. Indeed, in the past few decades, many compounds with strong antifouling activity have been isolated from marine sponges, corals, and algae[6,7,8,9,10].

The recent ban on toxic antifouling compounds has highlighted the need for the development of environmentally benignantifouling strategies and stimulated an active search for non-toxic natural marine antifouling compounds. A wide range of natural products have been screened for their potential to substitute the efficient but extremely toxic tributyltin (TBT), now banned in 27 countries[11]. Natural antifouling compounds from diverse organisms have been identified lately by their ability to inhibit biofouling[12]. This review attempts to bring together the bioprospection for natural antifouling compounds which would be potential candidates in the development of environmentally benign antifouling paints.

Antifouling compounds from sponges

Several studies showed the antifouling potential of sponge secondary metabolites in the Indian and Pacific oceans, Caribbean and Mediterranean seas[13,14,15,16]. Crude extracts of twelve sponges from north eastern and south eastern Brazil were tested against the attachment of the mussel *Pernaperna* through laboratorial assays, and promising species was highlighted[17]. The crude extract of four species significantly inhibited the attachment of byssus: *Tethyarubra, Tethyamaza, Petromicacitrina*, and *Hymeniacidon heliophila*. Chemical investigation of Indonesian marine sponges *Agelaslinnaei* and *A. nakamurai* afforded 24 alkaloid derivatives representing either bromopyrrole or diterpene alkaloids[18]. The agelasine derivatives inhibited settling of larvae of *Balanus improvisus* in an anti-fouling bioassay and proved to be toxic to the larvae.In a study reported by[19] thirty six species of sponges collected from the Gulf of Mannar, India, were screened for their inhibitory effect on fouling bacterial strains and cyprids of *Balanus amphitrite*. Of these, *Fasciospongia cavernosa* and *Petrosia nigricans* had high activity against the fouling bacteria and *Iotrochota baculifera* significantly inhibited larval settlement. *Oceanapia fistulosa*, *Haliclona exigua* and *H. cribricutis* exhibited considerable activity against both bacterial strains as well as barnacle larvae.

In addition to terpenoids, the pyrrole-imidazole alkaloids (PIA) have been extracted from several families of sponges with extensive focus of the bromopyrrole derivatives from the Agelasidae family. As natural products, the PIAs, especially oroidin 1, sceptrin 2, and bromoageliferin 3 have been found to be potent and toxic antifouling agents, working against microorganisms and higher organisms alike[20,21,22]. Bis-1-oxaquinolizidine alkaloids isolated from the marine sponge, *Haliclona exigua*, showed high inhibitory activity against the growth of seven strains of fouling bacteria as well as settlement inhibition activity against barnacle cyprids[23]. These compounds have been reported to have low toxicity and high therapeutic ratio.

The antifouling activity of a series of extracts and secondary metabolites from the epibiont-free Mediterranean sponges *Irciniaoros, I. spinosula, Cacospongia scalaris, Dysidea sp.,* and *Hippospongia communis* was investigated by Hellio et al., 2005. A number of the tested metabolites had antisettlement activity when assayed against barnacle, *B. amphtirite*, cyprids. Hydroquinone-C acetate and the *Dysidea* sp. alcohol extract, were noteworthy for reducing settlement at nontoxic concentrations. The defense roles and the antifouling activity of the organic extracts and the major metabolites of the sponges *Irciniaoros, I. variabilis* and *I. spinosula* has been reported by[24]. The dichloromethane extracts of *I. oros* and *I. spinosula* have shown promisinginhibition on the attachment rate of *Enteromorpha intestinalis*, *Ulvalactuca* and *Sargassum muticum* at 30 µg/mL. The ethanol extracts of *Ircinia oros* and *Ircinia spinosula* lead to the inhibition of the enzymatic activity of phenoloxidase which has a major role in the attachment of the macrofouler *Mytilus edulis.*

Antifouling compounds from marine algae

The antifouling potential of the marine microalga *Dunaliella salina* was investigated by Gao et al., 2014.Structural elucidation of the compounds have reported a series of unsaturated and saturated 16- and 18-carbon fatty acids The isolated purified extract was tested for antifouling activity, the EC_{50} value against *Skeletonema costatum* was 21.2 µg ml^{-1}, and the LC_{50} against *Balanus amphitrite* larvae was 18.8 µg ml^{-1}.Methanol and dichloromethane extracts of the seaweeds *Cladophora clavuligera and Sargassum wightii* were tested against five biofilm forming bacteria isolated from fouling test panels[25]. MeOH extract of *S. wightii* significant activity against *Pseudomonas* sp.1and *Bacillus* sp.2 whereas DCM extract showed activity against Micrococcus sp. *Pseudomonas* sp.1 and *Pseudomonas* sp.2. The seaweeds were tested moderately toxic to *Artemiasalina* by brineshrimp lethality assay.

The potential difference in the susceptibility of the two barnacle species (*Amphibalanus amphitrite* and *Semibalanus balanoides*) to antifouling substances obtained from algal extracts was tested by[26]. Most of the active extracts displayed activity towards *S. balanoides*, only few displayed targeted activity against *A. amphitrite*, or against both species.The results have been reported to highlight that surface extracts of algae displayed highest levels of activity than total extracts when tested on *S. balanoides*. Moreover, the difference illustrated that specific compounds in their ecological context can have potentially a better efficacy on target species. The antifouling activity of seaweeds collected from the Kollam coast was tested against a range of fouling organisms[27]. The extracts of *Laurencia brandenii* and *Lobophora variegata* recorded highest activity against biofilm forming bacteria. Besides, the extracts of *L. brandenii* and *Dictyota dichotoma* showed significant antifouling efficacy against *Mytilus edulis* and *Balanus Amphitrite.* GC-MS profile of *L. brandenii* has suggested that the purified fraction is primarily composed of octadecadienoic acid (49.75%) followed by "n-Hexadecanoic acid" (14.24%) which could have functional role in the chemical defense against fouling organisms.

From the brown alga *Lobophora variegata*, a new compound, lobophorolide has been identified. Lobophorolide, a 22-membered cyclic lactone has shown promising antifungal activities against marine fungi[28]. Methanolic extracts from *Padina tetrastromatica*, a brown alga from the Indian coast, have been found to inhibit several strains of bacteria, including *Pseudomonas vesicularis* and *Bacillus pumilus*, indicating its potential use as a future antifoulant[29].From the benthic marine macro-alga *Delisea pulchra*, halogenated furanones or fimbrolides, has been identified. This class of compound acts as a specific antagonist of the acylatedhomoserine lactone (AHL) regulatory system (quorum sensing) present in bacteria, thereby inhibiting bacterial colonization through a non-toxic and non-growth mechanism[30,31,32,33].

In a study reported by Chambers et al., 2011[34], crude ethanol extracts of the macroalgae *Chondrus crispus*, from both dried and fresh sources were tested and compared using bioassays based on five marine bacterial strains, five phytoplankton strains and two macroalgae to assess the antifouling efficacy. Macroalgae tests indicated that the extracts had an anti-germination activity 25-50 µg/mL against both *Undaria pinnatifida* and *Ulva intestinalis* spores. In the reported field trial, the antifouling coatings incorporating crude extract indicated reduced fouling coverage for the first 6 weeks, and slightly less weed fouling for up to 10 weeks as compared to control.

Aqueous ethanolic and dichloromethane extracts of thirty marine algae from Brittany coast were examined against 35 isolates of marine bacteria[35]. Among the 18 active non-toxic extracts, three were reported to show specific inhibition against only Gram-negative bacteria, 12 extracts showed inhibitory activities against only Gram-positive bacteria. Three extracts inhibited the growth of both Gram-positive and Gram-negative bacteria. Absence of toxicity on the development of oyster and sea urchin larvae and to mouse fibroblast growth suggests a potential for novel active ingredients in antifouling preparations. It has been also shown that the haloperoxidase systems present in the seaweed *Laminaria digitata* are capable of mediating the deactivation of acylatedhomoserine lactones in *Chromobacterium violaceum*, thus suggesting that haloperoxidase systems could potentially be used to control fouling[36].

Plocamium costatum, a red alga from the Tasmanian region of Australia, has shown a deterrent effect against the macrofouling barnacle, *B. amphitrite* at concentrations varying between 10 and 100 µg cm^{-2}[37]. Two biogenic compounds belonging to the halogenated monoterpene, have been isolated and purified from this alga, both of which deter fouling organisms at concentrations of 10 and 1 µg cm^{-2}, suggesting a possible role as natural antifoulants[38]. Phlorotannins, polymers of phenolicphloroglucinol (1,3,5-trihydroxy-benzene) isolated fromthe Australian brown algae *Ecklonia radiata* and *S. vestitum*, have been shown to inhibit the settlement and growth of propagules of the fouling green alga *Ulva* sp. at concentrations of 100 mg/L[39].Two non-polar secondarymetabolites, dictyol E and pachydictyol A,isolated from the brown alga *Dictyota menstrualis*, were found to inhibit the settlement of fouling barnacle larva,*Balanusneretina*[40].Elatol and deschlorelatol, two secondary metabolites isolated from the marine red alga *Laurencia rigida*, were found to exhibit strong deterrence against fouling invertebrate larvae derived from *Bugula neritina* and *Balanusamphitrite*[41,42].

Antifouling compounds from other marine macroorganisms

Antifouling activity of the crude extracts of two Red Sea puffer fish species, *Lagocephalus sceleratus* and *Amblyrhynchotes hypselogenion* were explored by[43]. The crude toxin extracts from the ovaries and skin mucus mixed with an inert simple paint formulation was applied to poly vinyl chloride plates and tested for coverage of fouling organisms. The study reported that extracts from *A. hypselogenion* showed better properties.Two benthic sea anemones (*Heteractis magnifica* and *H. aurora*) were tested for their antifouling activity against marine biofilm bacteria[44]. The crude extract of *H. magnifica* showed a maximum inhibition zone of 18 mm against *Pseudomonas sp.* and *Escherichia coli* and a minimum inhibition zone of 3 mm against *Pseudomonas aeruginosa*, *Micrococcus* sp., and *Bacillus cerens* for methanol, acetone, and DCM extracts, respectively. The butanol extract of *H. aurora* showed a maximum inhibition zone of 23 mm against *Vibrio parahaemolyticus*, whereas the methanol extract revealed a minimum inhibition zone of 1 mm against *V. parahaemolyticus*. Sesquiterpene isolated from the soft coral *Sinularia kavarattiensis* has been reported to exhibit considerable larval settlement inhibition properties against the cosmopolitan biofouler, *Balanus Amphitrite*[45]. Its low EC_{50} value and low toxicity has been highlighted for its enhanced potential for use as environmentally compatible natural product antifoulant.

Antifouling compounds from marine microorganisms

Most of the antifouling compounds have been isolated from seaweeds, sponges and other macroorganisms[46]with the disadvantage that such compounds are in limited supply. Recent investigations on this topic show that microorganisms in particular are promising potential sources of non-toxic or less-toxic antifouling compounds, as they produce a wide-range of potentially bioactive metabolites and also have the advantage of being easy to culture and to produce in large scale in short periods of time, easily ensuring product supply renovation for commercialization[47,48,49,50,51,52].Marine microorganisms provide a number of benefits including the possibility of genetic and chemical modification of the source organisms and compounds, respectively[53].

Antifouling compounds from marine bacteria

The organic extracts obtained from bacteria associated with the sponge *Aplysina gerardogreeni* when assayed against various microfouler strains (bacteria and microalgae) showed that 87% of bacterial extracts were active against the microfoulers tested[52]. Sixteen of them were reported to possess antifouling potential. The bacterial extracts also showed temporal variation. Active antifouling steroids isolated from the filamentous bacterium *Leucothrixmucor* showed antifouling activities against a representative soft fouling macroalgae (*Ulvapertusa*), a biofouling diatom (*Naviculaannexa*), and the bacteria *Pseudomonas aeruginosa* KNP-3 and *Alteromonas* sp. KNS-8 (Cho 2012). The chemical constituents having the antifouling activities were identified as 17-(1,2-dihydroxyl-5-methyl-hexane)-2,3-dihydroxyl-cholest-4-en-6-one and 13-acetate-17- (1,5-dimethylhexane)-cholest-7-en-3,5,6,15-tetraol

Marine bacteria *Pseudoalteromonas*, *Phaeobacter*, and *Vibrionaceae* isolated from Danish coastal waters have been reported to produce antifouling compounds[53]. Twenty-two of the marine bacteria that inhibited the growth of *V. anguillarum* were tested for their ability to prevent the adhesion of *Pseudoalteromonas* strain S91, and *Pseudoalteromonas* species were particularly effective in preventing bacterial adhesion. *Pseudoalteromonas* strains caused more pronounced inhibitory effects in the algal spore settlement assay than strainsbelonging to the *Vibrionaceae* and *Phaeobacter*. Bacterial symbionts of seagrass species *Thalassia hemprichii, and Enhalus acoroides* were tested for their antifouling activity[54]. Four bacterial symbionts capable of inhibiting the growth of biofilm-forming isolates were reported. Molecular identification based on 16S rRNA gene sequences revealed that the active bacterial symbionts belonged to the genera *Bacillus* and *Virgibacillus.*

The settlement of algal spores and invertebrate larvae of common fouling organisms in mariculture is affected by bacterial strains isolated from marine living surfaces. Bacterial strains isolated from the surfaces of seaweeds and invertebrates were tested for their effects on settlement of *Ulvalactuca* spores and *Hydroidesezoensis* larvae[55]. The strain CI4 identified as *Pseudoalteromonas* sp. The high proportions of host associated bacteria producing antifouling compounds suggest that these bacteria may help the host organism in the defense against fouling[56]employed the immobilisation of the antifouling bacterium *Pseudoalteromonas tunicata* in κ-carrageenan to demonstrate how a surface may be protectedfrom fouling by bacteria. Successful reduction of invertebrate (*Bugula neritina* and *Polysiphonia* sp.) larval settlement by the immobilized *P. tunicata* in κ-carrageenan beads has been reported.

Two compounds, 2-hydroxymyristic acid (HMA) and cis-9-oleic acid (COA), were isolated from a chloroform extract of the marine bacterium, *Shewanella oneidensis* SCH0402[57].HMA and COA either completely eliminated or decreased the optical density of fouling bacteria *Alteromonas marina* SCH0401 and *Bacillus atrophaeus* SCH0408 but the commercial, highly toxic antifouling tributyltin oxide (TBTO) never reduced the OD of the target bacteria by 100% even at higher concentration. Both HMA and COA inhibited germination of Ulvapertusa spores completely at 10 and 100 μg ml^{-1}, respectively, whileTBTO inhibited germination at 0.01 μg ml^{-1}.The potential of marine bacteria associated with soft coral *Sarcophyton* sp for the production of secondary metabolites against marine biofilm-forming bacteria was investigated by[58](Sabdono and Radjasa (2006). Six bacterial isolates were found to inhibit the growth of at least one of 7 biofilm-forming isolates. The most active strain USP3.37 was identified as *Pelagio bacter variabilis* by using 16S rDNA gene sequence analysis. Dark-green pigmented marine bacterium *Pseudoalteromonas tunicata* has been reported to produce antifouling agent[59] (Holmstrom et al., 1998). *P. tunicata* cells are dominated by monoenoic fatty acids (> 70%) which is typical of Gram-negative bacteria. The fatty acids 16: lω7c and 16:0 were the dominant fatty acids in both the cell and the supernatant extracts.

Antifouling compounds from marine fungi

A novel fungus *Aureobasidium pullulans* HN, isolated from marine biofilm has been investigated for production of antifouling compounds[60]. The fungal extract was reported to have antifouling activities. The EC50 of the extract against *Skeletonem acostatum* was 90.9 μg/ml, and its LC50 against *Balanus amphitrete* larvae was 22.2 μg/ml. The myristic and palmitic acids were found as the main toxicants by GC-MS.

Eighteen polyketides were isolated from the culture broth of a marine-derived fungal strain *Xylariaceae* sp. SCSGAF0086[61]. When tested against settlement of *B. neritina* larva two compounds showed strong antifouling activity and were considered as potential natural antifouling candidates based on their EC50 values. Three new 14-membered resorcylic acid lactones together with four known analogues were isolated from the culture broth of *Cochliobolus lunatus*, a fungus obtained from the gorgonian *Dichotella gemmacea* collected in the South China Sea[62]. Four of the seven tested compounds completely inhibited the larval settlement of B. Amphitrite EC50 values equal to or less than 5.0 μg/mL. One of the compound possessing the acetonide moiety, exhibited moderate antibacterial activity against *S. aureus* and only one compound showed moderate cytotoxicity.

Marine-derived fungus *Cladosporium* sp. F14 isolated from seawater has been exploredto produce antibacterial and antifouling compounds in the presence of glucose or xylose in nutrient enriched cultivation[63]. The EtOAc extracts of this fungus decreased larval attachment of the bryozoan *Bugula neritina and Balanus amphitrite*, and showed antibacterial activity on three types of tested bacteria. Antibacterial and antilarval benzene-type and diketopiperazine secondary metabolites were the compounds reported to be produced by the fungus.A sponge associated fungus, *Letendraea helminthicola*, has been reported to produce two antifouling compounds: 3-methyl-N-(2-phenylethyl) butanamide and cyclo(D-Pro-D-Phe)[64].Various parameters influencing the production of antifouling compounds and reported to be: temperature 18-30°C, salinity 30-45 ppt, pH 3.5-4.5. Carbon source: glucose and xylose, nitrogen source: yeast extract and peptone.

Antifouling compounds from actinomycetes

In a study reported by[65], 50 actinobacterial isolates were recovered from mangrove and estuarine sediment samples collected from Parangipettai and Pitchavaram coastal areas, Tamil Nadu and were phenotypically characterized. 42 out of 50 actinobacterial isolates inhibited one/more number of biofouling bacteria tested. Two actinobacterial isolates viz., strain PM33 and the strain PE7 showed promising activity against maximum number of biofouling bacteria tested. An active antifouling diterpenelobocompactol was isolated from marine actinomycete PK209 (identified as *Streptomyces cinnabarinus*) and its productivity was induced by co-culture competitor KNS-16 (identified as *Alteromonas*sp)[66] (Cho and Kim, 2012). The compound showed significant antifouling activity with EC_{50} value of 0.18 and 0.43 µg/ml against macroalga*Ulvapertusa* and diatom *Navicula annexa* respectively. Marine derived actinomycetes constituting 185 strains were subjected to screening for their antifouling activity[67]. One of the strain 291-11 (identified as *Streptomyces praecox* based on 16S rDNA sequence analysis) isolated from a sea weed *Undaria pinnatifida* was reported to have the highest activity. The chemical constituents representing the antifouling activity were identified as (6S,3S)-6-methyl-2,5-diketopiperazine and (6S,3S)-6-isobutyl-3-methyl-2,5-diketopiperazine and their therapeutic ratio > 15 claims it as a harmless antifouling agent.

In another study carried out by[68], extracts from six marine actinomycetes showed inhibition of biofouling bacteria in disc diffusion method. Extract from one of the strain (R1) showed maximum inhibition against all the fouling bacteria tested. The active compound was tentatively found to be sugar containing molecule and the potential actinomycete strain was identified as *Streptomyces filamentosus*. Eleven deep-sea *Streptomyces* isolates were screened for antifouling activity and *Streptomyces albidoflavus* strain UST040711-291 which was found to be one of the most active strains was cultured in large scale[69]. Five structurally similar compounds were isolated and identified from its crude extract and compared for antifoulng activity with four other structurally- related compounds isolated from another marine *Streptomyces* species. Three of the isolated compounds could inhibit the larval settlement of the barnacle *B. amphitrite*, a model fouling species, with low EC50 values but had small therapeutic ratios, suggesting they might have some toxicity as antifouling agents. Based on these findings a compound with a straight alkyl side-chain was synthesized and proved itself as a very effective non-toxic, anti-larval settlement agent against three major fouling larvae of *Balanus amphitrite, Hydroides elegans larvae* and *Bugula neritina.*

The marine bacterium *Streptomyces fungicidicus*, a new source of five bioactive diketopiperazines (DKPs) was isolated from Pacific sediment at the depth of about 5000 m[70]. Among the five DKPs that inhibited larval attachment of *Balanus amphitrite*, cyclo-(L-Val-L-Pro) had the highest antifouling activity. The compound showed a therapeutic ratioof 7.5 and the lowest EC50 and the highest LC50 which suggests that it has low toxicity towards to larvae and a low effective concentration.

Conclusions

Numerous antifouling compounds have been isolated from marine sponges, sea weeds, sea anemones, soft coral etc. But these compounds often have molecular structures that are too complex to be chemically synthesized. Excessive exploitation of these marine macroorganisms, more importantly the rare species, can lead to their extinction and will not provide a constant supply of antifouling compounds. In contrast, the supply of bioactive compounds from marine microorganisms will not be limiting because of the possibility of large scale fermentation for supply of these compounds. Marine microorganisms are excellent candidates for bioactive compounds with pronounced inhibitive effects on the settlement of both micro- and macro-fouling organisms. Unfortunately marine yeasts are unexploited for antifouling compounds. Reports on antifouling compounds from marine yeasts are almost nil. Thus it is clear from this review that research efforts could now be focused on bioprospecting of bioactives from marine yeasts.

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